



# *Seed Times*

Volume 12 No. 3, Sep - Dec 2019

The National Seed Association of India Magazine

*SEED AS A PLANT GENETIC RESOURCE*



# बीज बदलो... भाग्य बदलो....



शुरुआत बढ़िया होगी तो परिणाम अच्छा होगा ।



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# ABOUT NSAI

National Seed Association of India (NSAI) is the apex organization representing the Indian seed industry. The vision of NSAI is to create a dynamic, innovative and internationally competitive, research based industry producing high performance, high quality seeds and planting materials which benefit farmers and significantly contribute to the sustainable growth of Indian Agriculture.

The mission of NSAI is to encourage investment in state of the art R&D to bring to the Indian farmer superior genetics and technologies, which are high performing and adapted to

a wide range of agro-climatic zones. It actively contributes to the seed industry policy development, with the concerned governments, to ensure that policies and regulations create an enabling environment, including public acceptance, so that the industry is globally competitive.

NSAI promotes harmonization and adoption of best commercial practices in production, processing, quality control and distribution of seeds.

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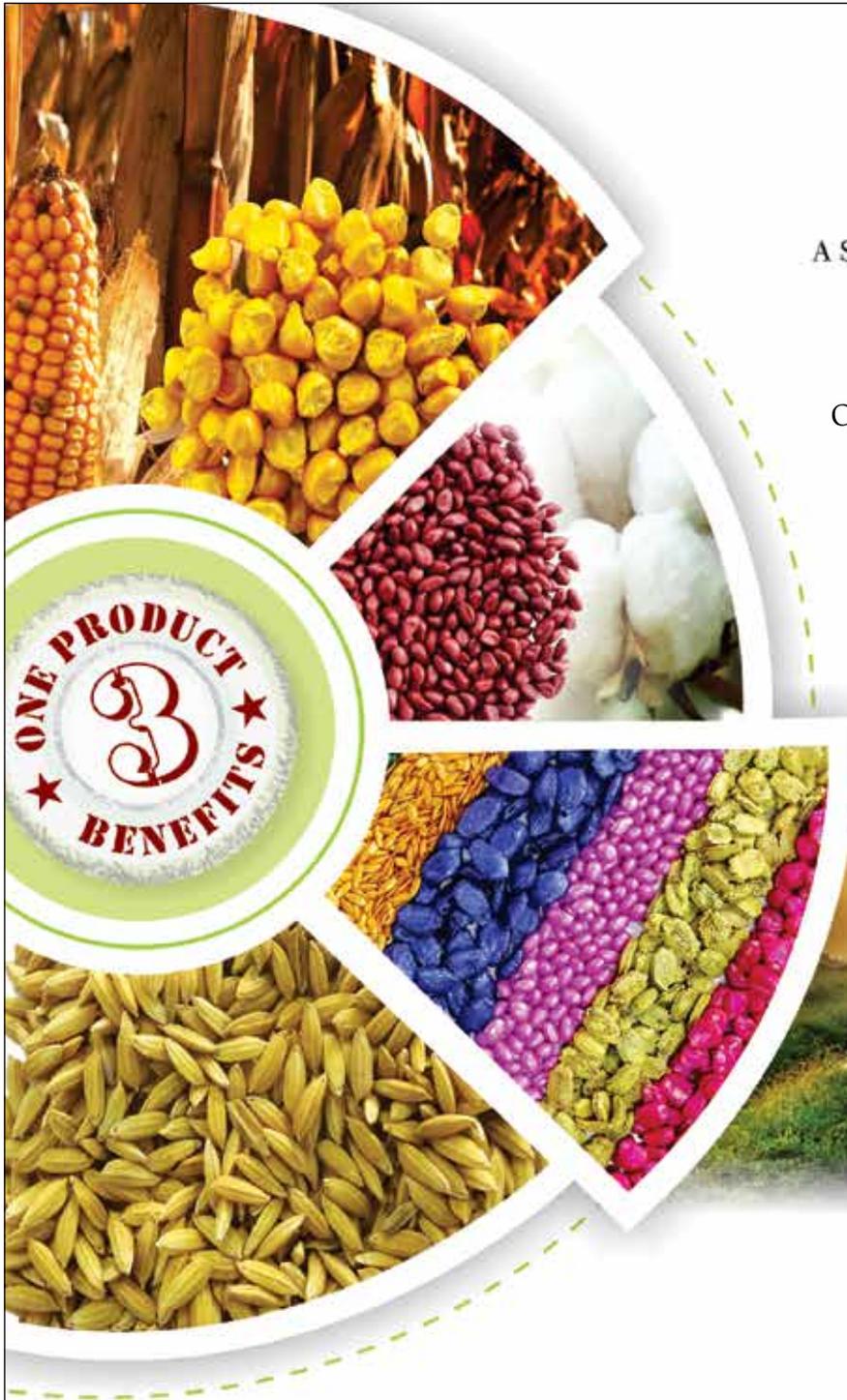
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# Message from Desk of President



The Indian gene Centre holds a prominent position among the 12 mega-gene centers of the world. A rich crop diversity is available in India in terms of both number of species and within the species. Landraces, traditional cultivars and farmer's varieties in several agricultural and horticultural plant species are abundant but a decreasing trend is noted in areas moving towards advanced agricultural practices.

Plant genetic resources (PGR) are the basic materials that are essential for development of improved crop varieties designed to combine high yield potential with superior quality, resistance to diseases and pests, and also better adaptation to abiotic stress environments. Their continued availability to plant breeders is necessary not only for sustaining advances in crop productivity but also for

stabilizing production in the country. These resources of known or potential use to man constitute a broad spectrum of diverse gene pools representing assemblage of landraces, primitive cultivars, varieties of traditional agriculture as well as wild and weedy relatives of crop plants.

I thank the contributors and team for bringing together this Seed Times on Seed as a Plant Genetic Resource. I hope you all read this and help in prevalence of genetic diversity provides great opportunity for crop improvement today and in distant future, when confronting situations would demand reconstruction of new cultivars and hybrids for sustaining higher production.

Happy reading!

**M Prabhakar Rao**





# Message from Desk of Executive Director



Germplasm collections are a wonderful treasure trove of genetic diversity and the foundation for all crop improvement programs. Seeds as a germplasm is the living genetic resources that is maintained for the purpose of plant breeding, preservation, and other research uses.

In recent years, many new plant species with desired and improved characteristics have started replacing the primitive and conventionally used agricultural plants. It is important to conserve the endangered plants or else some of the valuable genetic traits present in the primitive plants may be lost. Usually, seeds are the most common and convenient materials to conserve plant because many plants are propagated through seeds, and seeds occupy relatively small space. Further, seeds can be easily transported to various places.

In this edition of Seed Times, team NSAI brings some knowledgeable content on the theme Seed as a Plant Genetic Resource. This laudable objective will improve the well-being of our seed industry and farmers as well as ensure the modest way to contribute in achieving sustainable food production and food security to the nation.

I hope the readers would greatly be benefited from the magazine.

Happy Reading!

**R K Trivedi**







## Collection, evaluation, conservation and maintenance of seed spices germplasm

**A K Verma<sup>1</sup>, Kiran Kumari<sup>2</sup>, Ravi Y<sup>1</sup> and Gopal Lal<sup>1</sup>**

1. Scientists, ICAR- National Research Centre on Seed Spices, Tabiji, Ajmer-305206, Rajasthan

2. Assistant Professor, College of Horticulture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar - 385506, Banaskantha. Gujarat, India.

Seed spices are the group of plants that possesses the taste, flour and medicinal properties. Total 109 spices are listed by ISO and 63 spices are grown in India and out of which 20 are being classified as seed spices. Broadly, seed spices categorized into two groups based on the cultivation acreage; major and minor seed spices.

Seed spices are the group of plants that possesses the taste, flour and medicinal properties. Total 109 spices are listed by ISO and 63 spices are grown in India and out of which 20 are being classified as seed spices. Broadly, seed spices categorized into two groups based on the cultivation acreage; major and minor seed spices. Major seed spice crops are cumin, coriander, fennel, and fenugreek whereas ajwain, anise, caraway, celery, dill and nigella come under minor seed spices. India is blessed with a wide range of agroclimatic conditions from tropical to temperate zones, coastal plains to high altitudes and semi-arid to highly humid evergreen forests, therefore, it is an advantageous position to produce a number of seed spices. India



has also an old history of cultivation of spices and takes benefit of being the largest producer, exporter and consumer in the world. In India, seed spices are cultivated in an area of about 18 lakh ha and a production of about 18 lakh tonnes with the productivity of about 10 q/ha. The global demand for Indian Spices is increasing day by day. India is a consistent source of seed spices for importing countries worldwide. There has been ever increasing demand of seed spices and importing countries look at India for quality produce of seed spices. After the local consumption, India is exporting approximately 15 percent of its production annually and fulfills the 50-60 percent of world demand. The total export of seed spice crops is approximately 3700 crore (INR), out of which cumin alone contributes more than 2400 (INR) crore annually.

### Exploration and collection of seed spice germplasm

Exploration refers to collection trips and collection refers to tapping of genetic diversity from various sources and assembling same at one place. Seed spices have lack of variability for different essential traits. Therefore, a systematic work needed to increase the variability through native and exotic exhaustive exploration of germplasm. The germplasm of a crop species consists of various plant materials such as land races, primitive cultivars, released varieties, genetic stocks, wild and weedy relatives and mutants. Exploration is the purposeful collection of wild and cultivated plants in search of both primitive and advanced genetic materials that can be used to improve cultivated crops. Planning is required in dealing with germplasm collecting so that the explorer is in the right area at the right time and can search for and collect germplasm. During the exploration of seed spices, prior knowledge of the crops and species is very important. This includes agroecological zone of occurrence, diversity, distribution, maturity type and edaphic conditions etc. Based on needs and objectives of the exploration, mission can be for collection of the variability of a particular crop or broad based with the aim of capturing maximum diversity of different crops occurring in the same region and maturing at the same time. Thus, germplasm collecting task depends on the available genetic diversity in different area and crops. This includes, landraces/primitive cultivars, wild and weedy relatives of domesticated species, wild species used by man, wild species of potential use to man and obsolete and advanced cultivars. Extensive plant exploration is the old age plant selection on the basis of economic utility. Apart from indigenous collection, exotic germplasm from the Mediterranean and Western countries was also introduced and widened the genetic base of seed spices. The seed spice genetic enhancement through the collection of available germplasm is one of the basic breeding strategies followed. As India has endowed with nature, it has many types of seed spice germplasm from different regions of the country particularly from Rajasthan, Gujarat, North East, Himachal Pradesh and J&K.

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In germplasm collection process, site identification is the most important stage for the curator to design a core collection and is of great help in identifying duplicates. It is also essential information for eco-biological, evolutionary or population genetic research and for planning further collections.

Germplasm characterization consists of recording important yield attributing traits that determine the usefulness of a germplasm for a specific use in explicit conditions. After collection of germplasm, there is need for its systematic evaluation in order to know its various morphological, physiological and developmental characters.

Passport data of germplasm consists of all fundamental information recorded during the time of collecting the samples or the information provided by the sender regarding source/origin etc. Such information is very useful during all phases of genetic resources work. In germplasm collection process, site identification is the most important stage for the curator to design a core collection and is of great help in identifying duplicates. It is also essential information for eco-biological, evolutionary or population genetic research and for planning further collections. Records on topography or soil characteristics can be valuable for plant breeders too for improving adaptation to particular conditions or tolerance to edaphic or climatic stresses. The important passport descriptors are the site of collection (village/state/country), collector's number, type of material (population or pure line), date of collection, altitude, latitude and longitude for site of collection, status (wild, weedy, landrace, cultivar), growing conditions and source (field, farm store, institute, etc.).

### Germplasm evaluation/characterization

According to a recent International Board for Plant Genetic Resources (1985) definition, germplasm evaluation comprises the recording of those characters which are highly heritable, easily seen by the eye and are expressed in all environments. The characterization should provide a standardized record of readily assessable plant characters, together with passport data, go a long way to identify an accession. The role of germplasm in the improvement crop has been well recognized. However, until a collection has been properly evaluated and its attributes become known to breeders, it has little practical use.

Germplasm characterization consists of recording important yield attributing traits that determine the usefulness of a germplasm for a specific use in explicit conditions. After collection of germplasm, there is need for its systematic evaluation in order to know its various morphological, physiological and developmental characters. These include, productivity, crop duration, stress tolerance, disease and pest resistance and quality characters. The seed spices germplasm available at different centers like ICAR-National Research Centre on Seed Spices, Jobner, Jagudan, ICAR- Indian Institute of Sugarcane Research and other National Active Germplasm Sites NAGS for evaluation and utilization.

In the widest sense, the detailed evaluation of large collections requires a multidisciplinary approach, specific testing conditions involving disciplines of cytogenetics and evolution, physiology, pathology, entomology, biochemistry and agronomy. They all contribute information that bears on the choice and utilization of genetic resources by the breeders. Cytogenetic information is essential for the use of many wild relatives of crops. Such systematic and detailed evaluation of germplasm is more expensive and

time consuming but it has great value. In general, characterization and preliminary evaluation is done by the curator/germplasm scientists; further evaluation or detailed evaluation is mostly done by the breeders for taking additional information. Nevertheless, no hard and fast rule exists and the thorough germplasm evaluation can also be done by the crop curator in collaboration with plant breeders, pathologists, entomologists, agronomists and biochemists as per the requirements. Most of the seed spice crop varieties are released through selection from the germplasm (Singh and Solanki, 2015). There is lack of genetic resources with high volatile oil content in seed spices and resistance to biotic and biotic stresses (Malhotra and Vashishtha, 2007). The evaluation information on seed spices needs to be documented and catalog crop wise so that breeder/researcher can use it for improvement of seed spices.

### Germplasm holdings and maintenance

The exploration and conservation of germplasm is the prime objective of crop improvement. The total number of germplasms maintained by following centre in India (ICAR NRCSS, ICAR IISR, Sri Karan Narendra Agriculture University, Jobner, SDAU Jagudan, TNAU Coimbatore, HAU, Hisar, NDAUT Faizabad etc.) other than ICAR NBPGR, New Delhi. Germplasm holdings by different institutes in India is as under:

**Table 1: Total germplasm assemblage at NRC on Seed Spices, Ajmer, Rajasthan**

Crop	NRCSS Collection				NAGS Holding
	Indigenous	Exotic	Lost	Present available	
Cumin	100	7	-	107	247
Coriander	169	3	27	145	518
Fenugreek	82	59	6	135	733
Fennel	118	3	75	46	297
Ajwain	99	1	9	91	100
Dill	106	5	3	108	111
Nigella	21	3	-	24	24
Celery	36	-	-	36	36
Anise	18	-	-	18	18
Caraway	8	2	8	2	10
Total	759	83	128	714	2094

Source: NRCSS Annual Report 2015-16

**Table 2: Total germplasm assemblage at Centre for Research on Seed Spices, SDAU, Jagudan, Gujarat**

Crop	Exotic	Indigenous	Released	Total
Cumin	7	207	4	218
Fennel	4	41	3	48
Fenugreek	-	74	2	76
Coriander	22	84	2	76
Dil	-	77	3	80
Ajwain	-	60	1	61
Total	33	543	15	591

(Source: Tikka et al., 2011)

**Table 3: Total germplasm assemblage at SKN University, Jobner, Rajasthan**

Crop	Exotic	Indigenous	Total
Cumin	6	370	376
Fennel	20	161	281
Fenugreek	12	353	365
Coriander	102	753	855

(Source: Sastry and Singh, 2011)

All cultivated spices are cross pollinated except fenugreek, so maintenance is very tedious process.

In maintenance of germplasm, out crossing must be controlled with other varieties/lines. All cultivated spices are cross pollinated except fenugreek, so maintenance is very tedious process. In seed spices each germplasm is mostly grown every third years. To avoid the cross pollination, one meter uniform section of each row is covered with muslin cloth cage before flowering. The seed produced in cage serve as repetitive of the accession.

The system however suffers from the weakness like smaller number of plants sampled and low seed setting. Methods of obviate this weakness are in situ preservation, synthesis broad base gene pool/ gene reservoirs, long term storage and in vitro preservation etc. Previously reproduction behaviour of umbelliferous seed spices has been studied by several workers, found extent of cross pollination in coriander, fennel, ajwain. Further, it was reported that 2- 4.5 % cross pollination in cumin. It has been reported that coriander is an andromonoecious crop and it is highly cross pollinated and no inbreeding depression observed even after three generation of selfing. It was reported that up to 60 % out crossing may be recorded in closely spaced plants in coriander. Like coriander, cumin, fennel, nigella, ajwain, dill, celery, anise is cross pollinated and so maintenance of germplasm of these crops can be done by bagging of plants. The bagging should be done by using appropriate

size of butter paper or muslin cloth. Fenugreek, family fabaceae is self-pollinated crop hence, maintenance of fenugreek germplasm is very easy. The crop should be harvested at proper maturity stage to ensure the viability of seeds. Care must be taken during harvesting and threshing of germplasm to avoid mixing and germplasm to each other. The seeds after harvesting must be dried to an appropriate moisture level for the storage.

## Conservation of germplasm

After the collection of germplasm, its conservation is as much as important to germplasm collection. It is done by following methods:

**In-situ conservation:** Conservation of germplasm under natural conditions/native habitat is referred to as in situ conservation. This is achieved by protecting the area from human interference, such an area is often called natural park, biosphere reserve or gene sanctuary. All seed spices, are annual in nature and commercial propagation is done through seeds. Under normal storage condition the seeds of seed spices can be stored for two years without any loss in the viability. Thus, fresh seed of seed spices have to be produced once in period of three years. All seed spices are cross pollinated except fenugreek and pollination is carried out by honey bees. Therefore, bagging of plants by muslin cloth is essential. In each cage there should be 5 plant in case of fennel, dill, ajwain and 7-10 plants in case of cumin and coriander. The number is small to regenerate the individual accession but is enough for the maintenance of large germplasm by overcoming the problem of genetic drift.

**Ex-situ conservation:** Ex-situ conservation refers to conservation of germplasm away from its native habitat. Ex-situ conservation is generally a practical method of germplasm conservation. This method has benefits like it is possible to preserve entire genetic diversity of a crop species at one place, easy handling of germplasm and is a cheap method of germplasm conservation. In this method germplasm can be stored in seed bank, gene bank, tissue culture bank, botanical garden, cryopreservation etc.

## Documentation (germplasm cataloguing, data storage and retrieval)

Each germplasm accession is given an accession number. This number is prefixed in India, with either IC (Indigenous collection), EC (exotic collection) or IW (Indigenous wild). Information on the species and variety names, place of origin, adaptation and on its various features or descriptors is also recorded in the germplasm maintenance records. Catalogues of the collected germplasm for various crops are published by the gene banks and the huge amount of data recorded during the germplasm evaluation. Its compilation, storage and retrieval are now done using special computer programmes.

Care must be taken during harvesting and threshing of germplasm to avoid mixing and germplasm to each other. The seeds after harvesting must be dried to an appropriate moisture level for the storage.

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## Access and Sharing of **Plant Genetic Resources (PGR)**

**Vandana Tyagi and Pratibha Brahmi**

ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi-110012

**PGRs need to be exchanged and selected continuously for specific traits, to improve crops in terms of yield and nutritional value. Every nation is concerned with the acquisition of diverse and superior germplasm for conservation and utilization.**

Plant Genetic Resources (PGR) comprise of crop plants and their wild/weedy related species of actual or potential use. The development of improved types used today and those that would be cultivated in the future are essentially based on the effective utilization of PGR. These have helped in broadening the genetic base of crop plants within species and among species and in the diversification of cropping and farming systems through stability and sustainability. PGRs need to be exchanged and selected continuously for specific traits, to improve crops in terms of yield and nutritional value. Every nation is concerned with the acquisition of diverse and superior germplasm for conservation and utilization.

The exchange scenario has changed fast during the last decade, most evidently due to establishment of a mechanism to share equitable share in benefits, gained from the use of these resources. As a result, a paradigm policy shift came into existence with Convention on Biological Diversity (CBD), which



entered into force in 1993. CBD provides for the conservation and sustainable use of all biological diversity and establishes the process of the equitable sharing of benefits arising out of the use of biodiversity by reaffirming national sovereignty over genetic resources and stressed that the authority to determine access to genetic resources rests with the national governments subject to national legislation.

With CBD entering into force the access to PGR is now regulated to conserve, sustainable use and equitable benefit sharing. For access of plant genetic resources and equitable sharing of benefits policies and programmes are now well placed and regulated under different Acts and Treaties.

## Procedures for accessing Plant Genetic Resources

### From other countries Into India (Import into India)

For accessing any PGR from other countries, the Government of India has made it obligatory for all plant breeders and researchers to fulfill the two mandatory requirements as per the Plant Quarantine (Regulation of Import into India) Order, 2003 or PQ Order, 2003 ([www.plantquarantineindia.org](http://www.plantquarantineindia.org)). The two mandatory requirements are: 1) Import permit issued by ICAR-NBPGR before import of any material (IP) and 2) Phytosanitary certificate from the country of origin (PC). These two documents must accompany every seed/planting material consignment imported from abroad.

### Issuance of Import Permit

Director, ICAR-NBPGR has been authorized to issue an import permit for import of germplasm, transgenic or genetically modified organisms for research purposes and receive imported materials from custom authorities for its quarantine inspection and clearance and further distribution to the researchers in the country. Along with the application form PQ08, the processing fee for the issuance of IP should be sent. The fee is non-refundable. The recipient desirous of importing seed/planting material has to apply to the Director, ICAR- NBPGR on a prescribed application form (PQ Form 08). The IP is issued in form PQ 09 in triplicate. IP is valid for six months from the date of issue and valid for successive shipment provided the exporter and importer, bill of entry, country of origin and phytosanitary certificate are the same for the entire consignment. For private seed companies, there is a requirement that Research and Development of the firm is recognized by Department of Scientific and Industrial Research (DSIR) and the certificate is to submitted along with the PQ08 form, Commercial and bulk import is permitted based on the recommendations of EXIM Committee of Department of Agriculture, Cooperation and Farmers Welfare.

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## Phytosanitary Certificate (PC)

The second mandatory requirement is that of Phytosanitary certificate which is to be issued by the official agency of the donor country. Every consignment shall be accompanied by PC (original copy) issued by authorized officer at country of origin/ supplier country with additional declarations for freedom from specific pests and diseases as specified or that the pests specified do not occur in the country or state of origin as supported by documentary evidence thereof. PC is a document regarding the health status of consignment and issued by Government Official from the country of origin in the prescribed format of Food and Agriculture Organization (FAO). PC is also issued by the ICAR-NBPGR for all the germplasm material meant for export to foreign countries.

## Issuance of Import Permit for Transgenics

The provisions of PQ Order, 2003 are applicable to import of transgenic seeds as well. Department of Biotechnology (DBT), under Ministry of Science and Technology and the Ministry of Environment, Forests and Climate Change (MoEFCC) has a set of prescribed procedures for providing permission for import of transgenics. An indenter who wishes to import transgenics has to submit the proposal to Review Committee on Genetic Manipulation (RCGM) through the Institutional Biosafety Committee (IBSC). RCGM is an authorized agency of the Government of India, functioning under DBT, which assesses the applications submitted for importing transgenic material for research purposes and issues Seed Transfer Clearance Letter. RCGM examines the desirability of import of transgenic line, from the biosafety point of view. It includes representatives from DBT, Indian Council of Medical Sciences (ICMR), Indian Council of Agricultural Research (ICAR), Council of Scientific and Industrial Research (CSIR) and other experts in their individual capacity. After getting the technical clearance for import, from RCGM, the indenter has to apply to Director, ICAR-NBPGR, New Delhi for the issuance of IP in the prescribed PQ08 form along with Seed Transfer Clearance Letter issued by DBT.

**RCGM is an authorized agency of the Government of India, functioning under DBT, which assesses the applications submitted for importing transgenic material for research purposes and issues Seed Transfer Clearance Letter.**

**a non-Indian entity can access any biological resources or knowledge associated, for research, commercial utilization, bio-prospecting or bio-utilization, with the prior approval of National Biodiversity Authority (NBA) only.**

## Accessing Plant Genetic Resources from India (Export from India)

### Biological Diversity Act (BDA), 2002

Access to plant genetic resources from India is regulated under Biological Diversity Act (BDA), 2002. In compliance to Convention on Biological Diversity (CBD), Government of India enacted legislation Biological Diversity Act (BDA), 2002, and notified the Biological Diversity Rules, 2004. As defined in Section 3 (2) of the Act, a non-Indian entity can access any biological



resources or knowledge associated, for research, commercial utilization, bio-prospecting or bio-utilization, with the prior approval of National Biodiversity Authority (NBA) only.

Non-Indian entity is defined as (a) a person who is not a citizen of India; (b) a citizen of India, who is a non resident as defined in clause (30) of section 2 of the Income tax Act, 1961; (c) a body corporate, association or organization - (i) not incorporated or registered in India; or (ii) incorporated or registered in India under any law for the time being in force which has any non Indian participation in its share capital or management. Hence, all such persons or organizations need to seek approval of the Authority (NBA) for access to biological resources and associated knowledge for research or for commercial utilization and shall apply in the prescribed form ([www.nbaindia.org](http://www.nbaindia.org)). The application is now available online ( <http://absefiling.nic.in/NBA/login/auth>).



The access under collaborative research projects is however exempted under Section 5 of BDA, 2002 and the collaborative research project shall conform to the policy guidelines issued by the Central Government. ICAR-NBPGR facilitates the procedure for approval of export from the Competent Authority for requests received collaborative research projects.

### Facilitated Access under International Treaty on Plant Genetic Resources for Food and Agriculture

The Treaty provides for facilitated access to Contracting Parties for the crops of Annex I under Multilateral System (MS) solely for the purpose of utilization and conservation for research, breeding and training for food and agriculture and purpose that does not include chemical, pharmaceutical and/or other non-food/feed industrial uses. In case of multiple-use, their importance for food security should be the determinant for their inclusion in the MS and availability. It is also ensured that passport data and non-confidential descriptive information is to be made available along with the PGRFA.

Facilitated access is provided under a Standard Material Transfer Agreement (SMTA), adopted by the GB and conditions of the SMTA applies to the transfer of PGRFA to another person or entity, and subsequent transfers.

Intellectual property or other rights that limit the facilitated access of the PGRs are not to be claimed on resources accessed under the MS. However, PGRFA which is under development, will be made available at the discretion of its developer, during the period of its development. Facilitated access is provided under a Standard Material Transfer Agreement (SMTA), adopted by the GB and conditions of the SMTA applies to the transfer of PGRFA to another person or entity, and subsequent transfers. SMTA is a contract with standard terms and conditions and ensures that the provisions of the Treaty are followed by providers and recipients of PGRFA. Service charges details and quantity of seed permitted are depicted in Fig 1 and 2. The flow charts depicting the procedures of import and export are shown in Fig 3 and 4.

**Fig 1. Processing fee applicable for issuance of Import permit w.e.f. July 2017 (Fee to be paid as Demand Draft in favour of Director, ICAR-NBPGR payable at Delhi)**

S. No.	Organization/ Institution*	Import Permit	Fee amount in Rs.	GST @18%	Total Fee per permit in Rs.
1.	Public	Non Transgenic	200.00	36.00	236.00
2.	Public	Transgenic	350.00	63.00	413.00
3.	Private	Non Transgenic	450.00	81.00	531.00
4.	Private	Transgenic	700.00	126.00	826.00

#### Revalidation fee for Extension of validity or revision of Import Permit

S. No.	Category	Public Sector	Private Sector
1.	Revalidation of existing Import Permit for a further period of six months for import of non-transgenic germplasm	Rs. 50/-	Rs. 75/-
2.	Revalidation of existing Import Permit for a further period of six months for import of transgenic germplasm	Rs. 75/-	Rs. 100/-

#### \*Organizations/ Institution qualifying for import of germplasm/ research material

The government notifications recognizing the R&D status of an organization as issued by the Department of Scientific and Industrial Research/ Department of Science and Technology/ State Government Department (s), will be considered as the criterion for issuing the import permit to a given organization.

**Handling and Cargo Clearance charges for consignments received at the Airport**

Public Sector	Private Sector
Rs 2000 per consignment	Rs 4000 per consignment

**Quarantine Processing and Clearance Fee Applicable for all Samples Imported for Research Purposes\***

Category/ Plant Part	Public Sector		Private Sector	
	Non- transgenic	Transgenic	Non- transgenic	Transgenic
<b>1. Seed</b>				
Per sample	Rs. 150.00	Rs. 750.00	Rs. 300.00	Rs.1500.00
<b>2. Vegetative Propagules (VP)/ Tissue Culture tubes (TC)</b>				
One sample upto 10VP/10 tubes	Rs. 150.00	Rs. 375.00	Rs. 300.00	Rs. 750.00
For every additional VP/TC tube in a sample (11-100 VP/ TC tube maximum)	Rs. 15.00	Rs. 30.00	Rs. 30.00	Rs. 75.00
<b>3. Nursery/ Trial entries of cereals, grain legumes etc. ( not applicable for nurseries/ trials received from CGIAR Institutes)</b>				
Per sample/ replication)	Rs. 5.00	Not applicable	Rs. 20.00	Not applicable

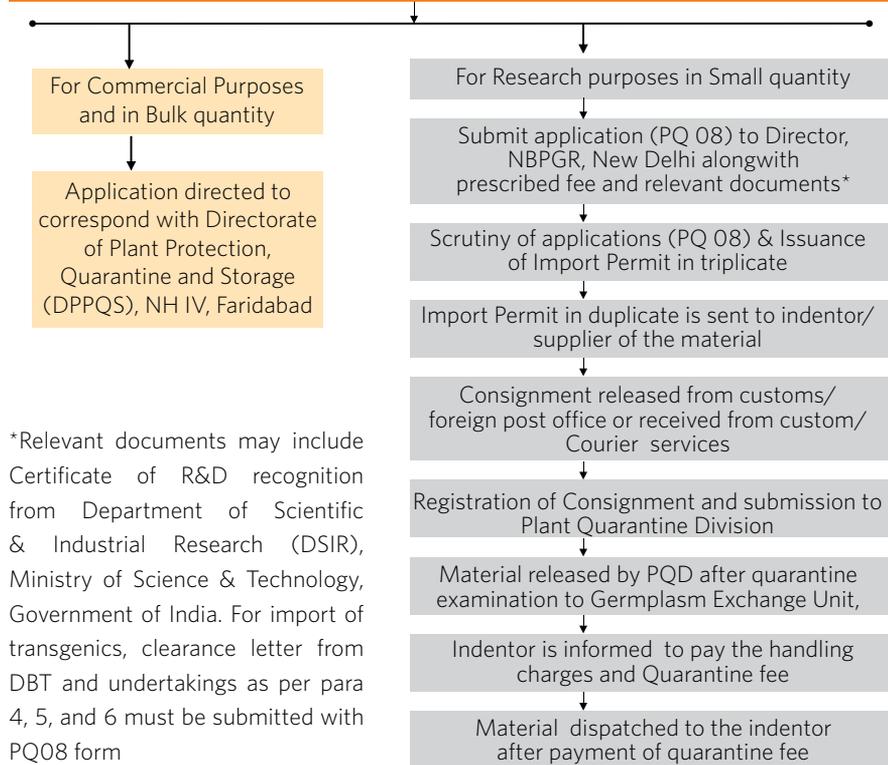
\* GST @ 18% applicable (rates as per prevailing Government orders)

**Fig 2: Maximum quantity permitted for Import and Export, per sample/ accession/ variety for research purposes**

S. No.	Plants parts	Quantity Permitted per sample/ accession/ variety
1.	Seed	
	<b>Large seeded crop species viz;</b> Zea mays, Helianthus spp, Carthamus spp. Phaseolus spp. Arachis spp., Dolichos spp., Mucuna spp., Pisum sativum, Cicer sp., Vicia spp., Cajanus spp., Canavalia spp., Palms and others	Up to 500g

S. No.	Plants parts	Quantity Permitted per sample/ accession/ variety
1.	Seed	
	Medium seeded crop species viz; Rice, Wheat, Barley, Oat, Lentil, Mungbean, Urdbean, Okra, Sorghum, Pearl millet and others	Up to 200g
	Small seeded crop species viz; Allium spp; Brassica spp; Capsicum spp; Solanum melongena, Carica papaya and others	Up to 100g
	Very small/ light weight seeded crop species viz; Tobacco, Tomato, Grasses, Eucalyptus and others	Up to 25g
2.	Vegetative Propagules	
	Number of rooted cuttings/ plants	Up to 25 in numbers
	Number of other vegetative propagules/ tissue culture tubes	Up to 50 in numbers

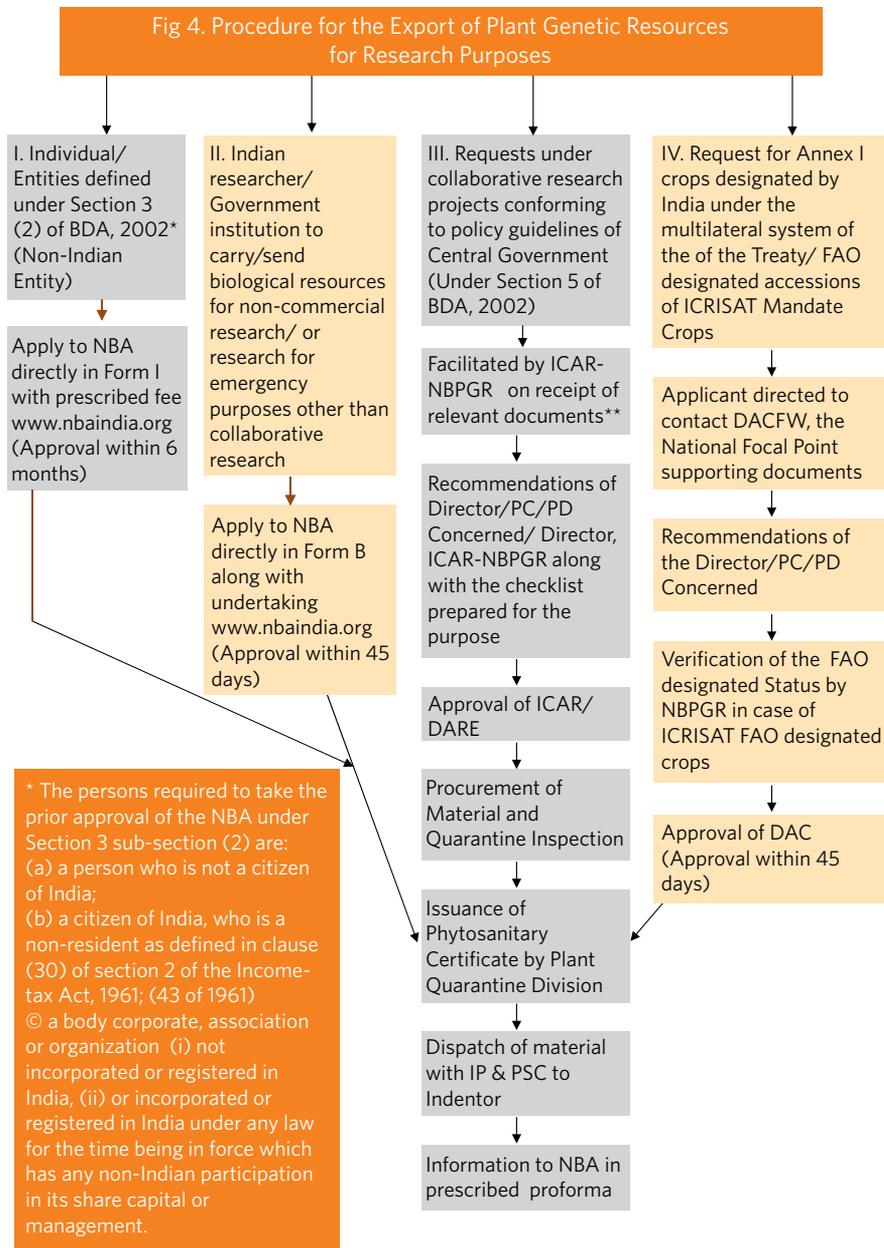
**Fig 3. Steps in Import of Germplasm as Per Plant Quarantine (Regulation of Import Into India), Order 2003**



\*Relevant documents may include Certificate of R&D recognition from Department of Scientific & Industrial Research (DSIR), Ministry of Science & Technology, Government of India. For import of transgenics, clearance letter from DBT and undertakings as per para 4, 5, and 6 must be submitted with PQ08 form



Fig 4. Procedure for the Export of Plant Genetic Resources for Research Purposes



**\*\*Documents :**

1. Details of the Collaborative Project as per MoEFCC Guidelines; 2. Request Letter; 3. List of Material; 4. EC/IC numbers; 5. Signed Copy of MTA; 6. Duly signed undertaking by both Pls

Private seed companies falling under Section 3 (2) of BDA, 2002 can access germplasm from NBPGR/NARS after the NBA's approval and signing of MTA.

For private companies that do not fall under Section 3 (2) of BDA, 2002 and are wholly Indian, they need to submit an undertaking on stamp paper that they do not fall under Section 3 (2) of BDA, 2002 and are wholly Indian.

Transfer of germplasm from one party to another involves Issues of ownership, Access, Use, Equitable sharing of benefits, Intellectual Property Rights, thus MTA/MAA defines the rights of the provider and the recipient with respect to the transfer of material.

## Access from National System (Supply within the country)

For accessing any germplasm of agri-horticultural crops from PGR stored/maintained by NBPGR/NAGS, the applicant is advised to submit the request in prescribed requisition preform for supply of seed/ planting material (GEX01) to the Director, NBPGR, Pusa Campus, New Delhi, along with duly filled and signed Material Transfer Agreement (MTA).

Private seed companies falling under Section 3 (2) of BDA, 2002 can access germplasm from NBPGR/NARS after the NBA's approval and signing of MTA.

For private companies that do not fall under Section 3 (2) of BDA, 2002 and are wholly Indian, they need to submit an undertaking on stamp paper that they do not fall under Section 3 (2) of BDA, 2002 and are wholly Indian. Further, they are required to submit DSIR Certificate that R & D of the company is recognized by DSIR. The private seed companies falling under Section 3 (2) of BDA, 2002 need to seek approval from NBA and on signing of MTA can contact NBPGR for the supply of the seed/planting material.

## Material Transfer Agreement (MTA) / Standard Material Transfer Agreement (SMTA)

A document which describes the conditions under which the transfer of material is made for specific use, addresses various issues such as ownership of the transferred material and its derivatives, liability, confidentiality and legally binding. They are contractual agreements used for transfer or acquisition of material.

Transfer of germplasm from one party to another involves Issues of ownership, Access, Use, Equitable sharing of benefits, Intellectual Property Rights, thus MTA/MAA defines the rights of the provider and the recipient with respect to the transfer of material. SMTA is a contract with standard terms and conditions approved by the Governing body of the Treaty and ensures that the provisions of the Treaty are followed by providers and recipients of PGRFA. Four forms of SMTA are in use namely Click wrap, Easy, Shrink-wrap and Hard copy MTA:

## Conclusions:

The obligations defined above, therefore, entail an appropriate national network to strengthen a single window system of exchange. As a single window system of exchange in India, NBPGR is regulating access of germplasm to other countries as per the current legal framework. It is continually serving the researchers/breeders/ other stakeholders by making available the diverse PGRFA.



# Gene bank and Germplasm Conservation

**Brijesh Mani Tripathi<sup>1</sup>, Priyanka Singh<sup>1</sup> and Vinay Chourasiya<sup>2</sup>**

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The future is uncertain. Nature deals with uncertainty through genetic diversity. While Agriculture deals with uncertainty through germplasm conservation and enhancement. But due to human activities, Plant Genetic resources (PGRs) are reducing in alarming rate up to 75% that underpins much of crop genetic improvement<sup>1</sup>. That is why there is a growing realization over the world that the introduction of modern agriculture has to be supplemented with measures to conserve germplasm for agriculture sustainability and environment adaptability.

Germplasm includes all the hereditary material i.e. all the alleles of various genes, present in the crop species (Breeding lines, cultivated species, obsolete varieties, landraces, special genetic stock) and its wild form or wild relatives. Germplasm collection in gene banks is one of the best methods to conserve the germplasm for short, medium and long term. Millions of germplasm accessions have been collected and conserved through in-situ and/or ex-situ method. A total of 28,027,770 accessions are being conserved world-wide by 446 organizations and in context of India, total 392,163 germplasm accessions

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are conserved by National gene bank (NGB) under National Bureau of Plant Genetic Resource (NBPGR). The major challenge now is how to collect and conserve the wild relatives, under-utilized crops and a number of native species that are in verge of extinction, and exploitation/utilization of this abundant collected resource that has extremely high bequest value. Hence, germplasm conservation should be extended out efficiently in the way to meet future challenges of food supply under a changing time, population and climate. And our responsibility towards future generations is unlocking the genetic potential stored in gene banks and its exploitation through genomics and breeding approach.

## Introduction

**Germplasm is the sum of the hereditary material i.e. all the allele of various gene, present in the crop species and its wild form or wild relative. It is also called as the plant genetic resource (PGR). It includes: Breeding lines, cultivated species, obsolete varieties, landraces, special genetic stock, Wild form and wild relatives.**

**Any loss in the genetic resource may cause irreversible loss in the unique resources narrowing society's scope to respond to new problem and opportunities to adapt in changing environment. So, germplasm conservation is exceptionally crucial for the sustainability and adaptability.**

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The plant genetic resources are reducing in alarming rate by rapid introduction of new high yielding and improved cultivars, overexploitation, habitat destruction, deforestation, population growth, genetic erosion, faulty and unscrupulous collection, climate change, less Research & Development. Recent reports have shown that loss of PGRs is up to 75% through human activities. It is faster over the past 50 years than ever before in human history<sup>1</sup>. And this is the worst news ever because it is the basic foundation of agriculture and ecosystem that ensure food/agriculture sustainability, that provide the basic raw material for crop improvement and enhancement, that helps us to adapt in changing environment and that, as a whole helps to sustain our life in this globe.

Any loss in the genetic resource may cause irreversible loss in the unique resources narrowing society's scope to respond to new problem and opportunities to adapt in changing environment. So, germplasm conservation is exceptionally crucial for the sustainability and adaptability. It has very high bequest value. It bridges the past and the future by ensuring the continued availability of genetic resources for research, breeding and improved seed delivery for a sustainable and resilient agricultural system.

And for the germplasm conservation, gene bank plays a key role in the conservation, availability and use of a wide range of plant genetic diversity for crop improvement for food and nutrition security maintaining future option open to cope with uncertain problems and challenges.



## Germplasm Conservation

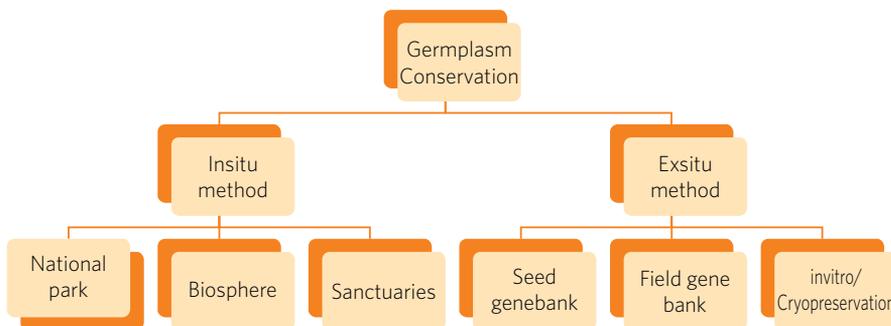
Under germplasm conservation, various activities are performed. They are: germplasm collection and conservation, evaluation, cataloguing i.e. data storage and retrieval, multiplication and utilization.

**a. Germplasm Collection:** It is the process of obtaining germplasm accession by exploration and/or procurement from other agencies usually from the center of origin and center of diversity. The species to be collected is prioritized on the basis of elite character, needs of the breeders and by the level of threat to the concerned species.

**b. Germplasm Conservation:** Then it is conserved in the gene banks by two method/approach, in-situ and ex-situ. Conservation of germplasm in its natural habitat is the in-situ conservation like in national parks, biospheres reserve or gene sanctuary (example gene sanctuaries in the Meghalaya for citrus). Similarly, ex-situ conservation is the conservation of the germplasm away from its habitat.

The ex situ conservation of plant genetic resources started by the mid-twentieth century as a reaction to the rapid loss of agricultural biodiversity. Ex-situ germplasm conservation can be achieved by three types of gene bank namely: seed banks, field gene bank and in vitro/cryopreservation gene bank.

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### 1. Seed gene bank:

Here, the germplasm accessions are stored as seed. Different seed with different purpose and requirement are stored in different environment. For example,

On the basis of their storability, the seeds can be categorized as below:

1. Orthodox seed (desiccation-tolerant): It can be dehydrated to low water content and are responsive to low temperatures without losing their viability. So, it is stored below 5% moisture content.

2. Recalcitrant seed (non-orthodox): The viability of the seeds lowers drastically if their moisture content is less than 12-30%. So, it requires other method of ex-situ conservation like field gene bank, tissue bank, etc.

On the basis of use of germplasm collection, it can be categorized as under:

S. No	Collection	Time (years)	Temp	Moisture content	Purpose/use
1.	Base Collection	50-100	-200 C	5%	For long term storage & used only when the other germplasm source is unavailable
2.	Active Collection	8-10	00C	8%	Evaluation, multiplication and distribution of an accession
3.	Working Collection	3-5	5-100C	8-10%	For frequent utilization by breeder for crop improvement

## 2. Field gene bank:

It is grown in the field, under natural condition especially essentially for the recalcitrant seed, vegetatively propagated or apomictic crop species. However, it suffers from the serious limitation like large area requirement, expensive/difficult management, disease and insect attack, and climate factor.

## 3. In vitro/ Cryopreservation gene bank:

Cryopreservation is the storage of biological materials (seeds, plant embryos, shoot tips/meristems, cell and/or pollen, DNA) at ultra-low temperatures, usually that of liquid nitrogen (LN) at -196 °C. Under these conditions, biochemical and most physical processes are halted and materials can be conserved over the long term. These modes of conservation constitute a complementary approach to other modes and are necessary for safe, efficient and cost effective conservation.

**c. Evaluation and Cataloguing:** The collected germplasm should be evaluated for their feature and traits. The characters assessed are related to the need of the breeders and other uses. It is most critical step to prepare catalogue and utilization of a collection.

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#### d. Utilization:

The overall aim of the germplasm conservation is utilization of the germplasm to meet present as well as future needs and demand. The germplasm is directly released as a variety, subjected for developing as variety or used as parents in hybridization in a breeding programme.

### Status of germplasm conservation in World

A total of 28,027,770 accessions are being conserved worldwide by 446 organizations represented in Genesys; of these, 3.78% (100,607) are Indian-origin accessions. Globally, the Consultative Group on International Agricultural Research (CGIAR) centers established 11 gene banks. In addition to this, about 1,750 individual gene banks are reported worldwide; out of which 130 gene banks hold more than 10,000 accessions and 8 have more than 100,000 accessions<sup>1</sup>. The major depositors of germplasm worldwide are SGSV (Svalbard Global Seed Vault), ICRISAT (the International Crops Research Institute for the Semi-Arid Tropics), IRRI (International Rice Research Institute), USDA (United States Department of Agriculture), ICARDA (International Center for Agriculture Research in the Dry Areas), CGIAR (Consultative Group for International Agricultural Research), etc.

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### Status of germplasm conservation in India

In context of India, it is one of the 12 world mega biodiversity centers and three of the 34 Hot Spots of Biodiversity, contributing 8.25% to the global biodiversity, 2018. This clearly shows India is floristically and genetically rich nation. However, loss of single part of germplasm may cost expensive as no single individual of any one species contains all the genetic diversity for that species. And genetic potential is represented by diversified germplasm to adapt to changing environment. There are many evidences showing the importance of single gene from India in saving crops from epidemics. For example, a gene transferred from *Oryza nivavra* from Odisha saved rice crop against the growth-stunting virus in Indonesia, single gene for downy mildew resistance in muskmelon crop, green bug resistance in sorghum in the United States had resulted in millions of dollars of annual benefit to America. Recently in rice, Sub1A (from FR13A) and PSTOL1 (from Kasalath) are being used globally to save rice from losses due to flooding and improving P use efficiency.

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Such and several other gene rich PGRs exist in India. So for, the germplasm conservation, a nodal agency National Bureau of Plant Genetic Resource (NBPGR), was established in 1976 by the Indian Council of Agricultural Research (ICAR), New Delhi. It is the third largest gene bank in national level involving 12 regional stations located in the diverse agro climatic zones of the country holding total 392,163 germplasm accessions. NBPGR houses national gene bank (NGB) that maintain germplasm in seed banks, field bank, slow culture and also in the cryopreserved accession (Singh, 2017).

Among 12 module of NGB, one module is used for medium-term storage with 19,556 accession and other 11 module are used for long term storage with of 3,39,194 accession of germplasm of various crop field and medicinal, agroforestry and aromatic plants by the end of 2006(Singh, 2017). Among them,1,81,325 accessions of different crops characterized and evaluated and 80 crop catalogues was developed.

Also, noble germplasm is collected like Carotenoid-rich cucumber from Manipur and Mizoram, Brown-netted cucumber (*Cucumis sativus* var. sikkimensis) cold tolerant from Meghalaya, Tree cotton (*Gossypium arboreum*) with long boll (11.1 cm) from Mizoram, Light brown-linted (naturally colored) tree cotton (*Gossypium arboreum*) from Tripura, etc.

**Focus of Expolrations undertaken**

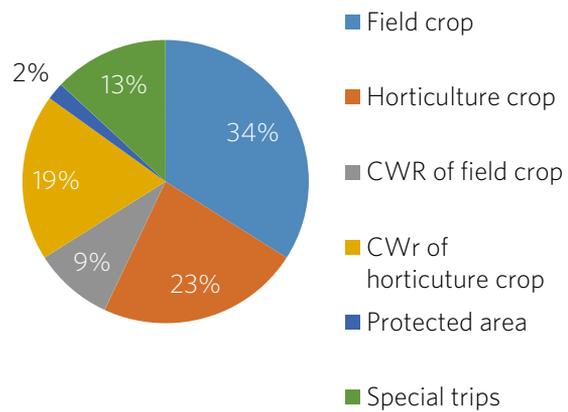
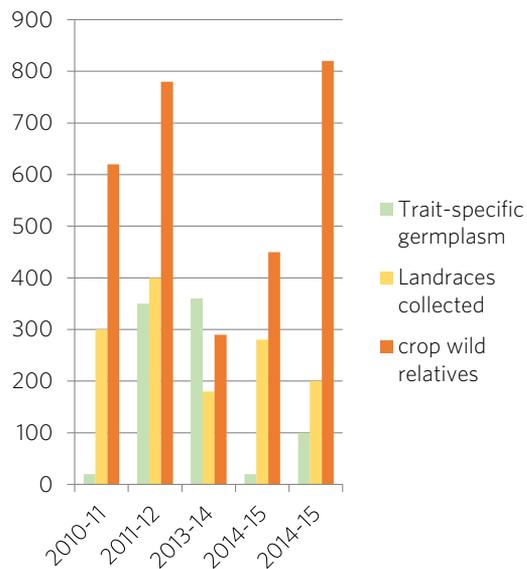


Chart: Focus of exploration undertaken



Graph: Germplasm collected during 2010-15



## Problem and limitation

Though, ex situ germplasm collections in the gene bank have increased enormously in number and size over the last three to four decades, its conservation is under question now. In particular this is due to the ever rising costs of maintenance, utilization, regeneration and the subsequent possibilities for genetic erosion occurring in a genebank<sup>4</sup>. Also, in spite of urgent need and unquestioned value of germplasm collection and conservation, only less than 30% of the countries have formal national germplasm conservation programme (Singh, 2017).

Still many wild relatives, under-utilized crops and a number of native species that occur in the wild are poorly represented in most of the ex situ collections and require greater attention as they are in verge of extinction. Therefore, the emphasis should be on proactive management of gene banks by adopting a complementary approach, and striking an optimal balance between scientific considerations, available personnel, infrastructural and financial resources under prevailing conditions.

## Conclusion

In today's world there is significant pressure to improve agricultural production by developing food crops that not only adapt to environment changes but also meet the growing food demand of constantly growing population. To meet increasing demands of our population there is an ominous conflict between agricultural modernization to optimize production, and the preservation of indigenous agriculture along with the genetic diversity to meet the future challenge. So, to meet future challenges of food supply under a changing time, population and climate - Germplasm conservation in gene bank is the best solution. However, it should be promoted more efficiently and utilized using genomic and breeding approaches for enhancing the agriculture sustainability and environment adaptability.

Beside dependency on gene bank, every single individual should be responsible in their own way for the conservation of the germplasm through practice of multiple cropping, priority to landraces, Community Biodiversity Management (CBM), Integrated Farming System, reduction of agrochemical use, avoidance of deforestation, etc.

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# Plant Genetic Resources: Conservation, Evaluation, Exchange and Way Forward"

**S K Abdus Samad**

Kalyani Seed Farms Pvt. Ltd.

## Introduction

Genetic resources are simply which have the potential value, it consists diversity of seeds of traditional varieties, modern cultivars and other plant species. Plant genetic material are the biological basis of food security and directly or indirectly support the livelihoods of every person on the earth. Seeds and plant genetic resources are interrelated with each other in agro ecosystem. When seeds are produced it can store as germplasm for conservation of plant genetic resources in agro ecosystem.

Plant genetic resources dates to more than 10000 years ago, when farmers selected from the available genetic variation, they found in wild species to develop their plants. Germplasm are living genetic resources such as seeds or tissues that are maintained for the purpose of plant breeding. Vavilov first

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Vavilov first called attention to the potential of crop relatives as a source of novel trait variation for crop improvement (Vavilov,1926,1940). The role of germplasm banks is to collect, maintain, preserve and distribute seeds representing the genetic diversity of crop species.

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### Germplasm Conservation

As following Second World War, the Food and Agriculture Organization of the United Nations became the main organization promoting the conservation of Plant Genetic Resources. Its consultative group on International Organization after that IBPGR (International Bureau of Plant Genetic Resources) has established.

Gene banks were created by the middle of the twentieth century to preserve cultivated biodiversity when landraces began to be substituted by modern varieties. This move was generally accepted as a necessary step to safeguard the future. After about 75 years of collecting and maintaining genetic resources, the increasing ability of biotechnology to create new variability brings the roles of gene banks in the present and near future into question. As a continuation of several workshops that started in 2014, staff of some representative gene banks have met to discuss how the Spanish Plant Genetic Resources Network can be improved, identifying the following major shortcomings: lack of efficient coordination in the distribution of species among gene banks; too many gene banks; existence of detected and undetected duplicates; insufficient rate of regeneration; insufficient phenotyping, genotyping, and epi phenotyping; unsatisfactory rate of use by end users; and, insufficient funding.

### Exchange of Germplasm

The ICRISAT-PQL, in conjunction with NBPGR Regional Station, Hyderabad, conducts seed health tests on germplasm prior to export. Pre-export inspection of seed multiplication fields by NBPGR quarantine officials for seed borne diseases at various growth stages of the crop to avoid their spread.

Protocol of Germplasm exchange is as given below:

- Collection of seed from fully mature and healthy plants.
- Cleaning seed to remove insects, pathogen propagules (smut sori, ergot sclerotia, and nematode cysts), weed seed, crop debris, soil clods, stones, other foreign material, and small, shrunken, discolored and damaged seed.
- Submission of an on-line request for export of germplasm (form available at ICRISAT intranet under GT-Crop Improvement).



- Submission of untreated seed in fresh muslin bags or paper packets along with the four-point declaration certificate (available at PQL) for quarantine processing.
- Submission of the importing country's plant quarantine requirements, such as import permit, non-commercial value certificate, additional declaration for seed borne pathogens and pests, and any other specific regulations/requirements (ref- plant quarantine guideline and procedures for germplasm exchange of ICRISAT mandate crops by ICAR).
- Ensuring that appropriate genetic resources with relevant traits are available and accessible is crucial for food security. Globally, the issue of access and benefit-sharing (ABS) is addressed, in varying degrees of detail, by the Convention on Biological Diversity (CBD), the FAO International Treaty on Plant Genetic Resources for Food and Agriculture and the Nagoya Protocol, a supplementary agreement to the CBD (ref- FAO.org).

## Way Forward

As a considerable increase in public funding is unlikely, we propose some strategies to increase the efficiency of the system. The most urgent tasks are to strengthen the rationalization of the network by establishing a clear hierarchy and functions, to improve the information in the base collection by deep characterization including not only phenotypes but also uses and utilities, to progressively replace the active collections with focused core collections constructed to meet users' needs, to optimize regeneration protocols, to limit new collecting expeditions of Spanish crop wild relatives to those growing in threatened habitats, and to develop user-friendly platforms to access germplasm documentation, including a unified system of descriptors and classification categories.

Current advances in biotechnology, and especially those in gene editing will have without doubt an impact on the role of genebanks. However, the high number of genes and gene combinations created by evolution they hold cannot be produced by these techniques at present. So, these reservoirs of variability will continue to be indispensable for the near-medium future while the function of all the genes is unveiled. In turn, biotechnologies and gene editing will allow us to take advantage of the information held in genebanks in a more efficient and fast way, contributing to a better rationalization and functioning.

The ex situ conservation of plant genetic resources started by the mid-twentieth century as a reaction to the rapid loss of agricultural biodiversity, mainly due to the replacement of landraces by improved varieties (Gepts, 2006; Van de Wouw et al., 2009; Khoury et al., 2014). This replacement was made possible

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by enormous energy inputs into agrarian systems in the form of machinery, fertilizers, pesticides, herbicides, irrigation, protected cultivation, etc., which make environmental conditions more uniform, thereby allowing a limited number of improved varieties to be grown everywhere, replacing landraces adapted to microenvironments, local cultivation methods, and cultural elements of use. It has been estimated that 70% of currently cultivated crops are of foreign origin, while the traditional crops indigenous to each area are disappearing (Khoury et al., 2016).

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## Quarantine Processing of Plant Genetic Resources under Exchange

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Plant quarantine is a government endeavor enforced through legislative measures to regulate the introduction of planting material, plant products, soil and living organisms, etc. in order to prevent inadvertent introduction of pests (including fungi, bacteria, viruses, nematodes, insects and weeds) harmful to the agriculture of a country/ state/ region, and if introduced, prevent their establishment and further spread (Kahn et al., 1989).

The historical Irish famine of 1845, caused by late blight of potato (*Phytophthora infestans*) introduced from Central America; powdery mildew (*Uromyces blaschkeanus*), root eating aphid (*Phylloxera vitifolia*) and downy mildew (*Plasmopara viticola*) of grapes into France in quick succession in mid 19th Century from America; coffee rust into Sri Lanka in 1875 and its subsequent introduction into India in 1876 are prominent examples that clearly demonstrate that introduction and establishment of quarantine pests into new areas can severely damage the crop production and economy of a region/ country (Khetarpal et al., 2006). Likewise, in India also, a number of exotic pests got

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ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR is the nodal agency for PGR management in the country, has been empowered under the PQ Order for issuance of Import Permit and to undertake quarantine processing of all imported PGR including transgenics and trial material meant for research. Besides, NBPGR also tests samples of bulk imports sent by DPPQS and its Regional Plant Quarantine Stations for presence of exotic pests.

introduced along with imported planting material causing serious crop losses from time to time. These include the recently introduced fall army worm *Spodoptera frugiperda* in maize and rugose spiralling whitefly (*Aleurodicus rugioperculatus*) in 2018; root knot nematode of Guava *Meloidogyne enterolobii* in 2017; *Puccinia horiana* causing white rust in chrysanthemum in 2016; tomato pin worm *Tuta absoluta* in 2014, Jackbeardsley mealybug (*Pseudococcus jackbeardsleyi*) in 2012, papaya mealy bug (*Paracoccus marginatus*) in 2007 are recent and glaring examples that clearly demonstrate that introduction and establishment of quarantine pests including weeds into new areas can severely damage the crop production and economy of a region/ country (Dubey and Gupta, 2016). These introductions highlighted the fact that increased international travel and trade had exposed the country to the danger of infiltration of exotic pests harmful to our agriculture.

With the liberalization of trade under World Trade Organization (WTO), the quarantine set-up including legislation and infrastructure of the country has been reviewed. As far as legislation is concerned, the Destructive Insects and Pests (DIP) Act was legislated by the British government ruling India in 1914 which was retained revising it as per requirements over the years through various amendments. However, after the WTO came into force, India legislated the Plant Quarantine (Regulation of Import into India) Order in 2003, henceforth referred to as the PQ Order. The Directorate of Plant Protection Quarantine and Storage (DPPQS) of the Ministry of Agriculture and Farmers Welfare is the nodal agency for implementation of PQ Order. ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR is the nodal agency for PGR management in the country, has been empowered under the PQ Order for issuance of Import Permit and to undertake quarantine processing of all imported PGR including transgenics and trial material meant for research. Besides, NBPGR also tests samples of bulk imports sent by DPPQS and its Regional Plant Quarantine Stations for presence of exotic pests.

ICAR-NBPGR is well equipped with most modern quarantine facilities including a Containment Facility of Level 4 (CL- 4) for quarantine processing of transgenic germplasm in a risk-free manner. ICAR-NBPGR also has a well-equipped quarantine station at Hyderabad, which mainly deals with the quarantine processing of PGR meant for Southern India including State Agricultural Universities, ICAR institutes, private industry and international institutes viz., International Crop Research Institute for Semi-arid Tropics (ICRISAT), CIMMYT and AVRDC.

### Plant Quarantine: Legislation

The quarantine measures are of utmost relevance to a country like India whose economy is largely agriculture based. The awareness to quarantine measures in India started in early 20th century when the Indian Government in 1906 ordered compulsory fumigation of imported cotton bales to prevent

introduction of Mexican cotton boll weevil (*Anthonomus grandis*). With a view to restrict the entry of exotic pests, pathogens and weeds through regulation of imports, the Government of India legislated the Destructive Insects and Pests (DIP) Act in 1914 ([http://plantquarantineindia.nic.in/pqispub/docfiles/dip\\_act.htm](http://plantquarantineindia.nic.in/pqispub/docfiles/dip_act.htm)). This Act has been amended through various notifications issued from time to time also restricted the movement of certain planting material from one state to another state within the country through domestic quarantine. In 1984, a notification was issued under this Act namely Plants, Fruits and Seeds (Regulation of Import into India) Order popularly known as the PFS Order which was revised in 1989 after the announcement of the New Policy on Seed Development by the Government of India in 1988, proposing major modifications for smooth quarantine functioning. This Order has now been superseded by the Plant Quarantine (Regulation for Import into India) Order 2003 which came into force from April 1, 2004 as there was an urgent need to fill-up the gaps in existing PFS order regarding import of germplasm/ GMO's/ transgenic plant material/ bio-control agents etc., to fulfill India's legal obligations under the international Agreements, to protect the interest of the farmers of the country by preventing the entry, establishment and spread of destructive pests, and to safeguard the national bio-diversity from threats of invasions by alien species. Under this Order, the need for incorporation of Additional/ Special declarations for freedom of import commodities from quarantine and invasive alien species (IAS), on the basis of standardized pest risk analysis (PRA), particularly for seed/ planting materials is also dealt with. Further, the scope of plant quarantine activities has been widened with incorporation of additional definitions. The other salient features of the Order are:

- Prohibition on import of commodities with weed/ alien species contamination as per Schedule VIII; & restriction on import of packaging material of plant origin unless treated.
- Provisions included for regulating the import of soil, peat & sphagnum moss; germplasm/ GMOs/ transgenic material for research; live insects/ microbial cultures & biocontrol agents and import of timber & wooden logs.
- Agricultural imports have been classified as (a) prohibited plant species (Schedule IV); (b) restricted species where import permitted only by authorized institutions (Schedule V); (c) restricted species permitted only with additional declarations of freedoms from quarantine/ regulated pests and subject to specified treatment certifications (Schedule VI) and; (d) plant material imported for consumption/ industrial processing permitted with normal Phytosanitary Certificate (Schedule VII).

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- Additional declarations being specified in the Order for import of 698 agricultural commodities with specific lists of more than > 1000 quarantine pests and 57 weed species.
- Notified points of entry increased to 130 from the existing 59.
- Certification fee and inspection charges have been rationalized.

So far, i.e., till December 2019, 77 amendments of the Plant Quarantine (PQ) Order 2003 have been notified to the WTO revising definitions, clarifications regarding specific queries raised by quarantine authorities of various countries, with revised lists of crops under the Schedules IV, V VI and VII.

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### National Plant Quarantine Set-up

The Directorate of Plant Protection, Quarantine and Storage (DPPQS) of Ministry of Agriculture and Farmers Welfare is the apex body for implementation of plant quarantine regulation and the PQ order forms the basis of the functioning of the Directorate. It has a national network of 59 plant quarantine stations at different airports (13), seaports (34) and land frontiers (12). The administrative structure of plant quarantine is shown in the organizational chart (see figure 1).

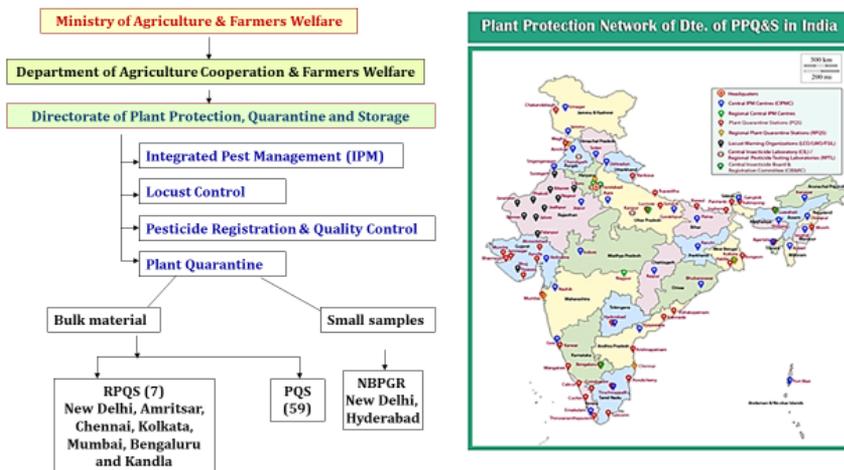


Figure 1: DPPQS Organizational Chart

In all, three categories of materials are being imported: (a) bulk consignments of grains/ pulses for consumption, (b) bulk consignments of seeds/ planting materials for sowing/ planting, and (c) samples of germplasm in small quantities for research purposes. The PQ Stations under the DPPQS undertake quarantine processing and clearance of consignments of the first two categories. However, ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR) undertakes the quarantine processing of all plant germplasm and transgenic planting material under exchange. It has developed well-equipped laboratories and green house complex (Bhalla et al., 2018). A containment facility of CL-4 level has been recently established for processing transgenics (Gupta et al., 2007).

### Procedure for Import of PGR

Research institutions of public and private sector interested in importing plants or planting material should request ICAR-NBPGR for Import Permit (IP), which is not transferable. Phytosanitary Certificate is also a statutory requirement issued by the country of export and is a proof that the consignment has been examined according to the requirements of the importing country and found to be free from the quarantine pests. On arrival of the plant material, they are carefully processed. In case material is found to be infected/ infested with pests, all efforts are made to salvage the material. Only in cases, when the material cannot be salvaged it is incinerated. In case post-entry quarantine (PEQ) examination of the imported material is required, it is done at PEQ greenhouse facilities, at NBPGR, New Delhi, its Regional Station, Hyderabad and ICRISAT and also at the indenter's PEQ growing facility, if mentioned in the IP application form.

### Methods for Detection and Interception of Pests in Quarantine

ICAR-NBPGR imports every year ~ 100,000 samples of germplasm and international trial material for research both by public and private sector. The Division of Plant Quarantine at ICAR-NBPGR has developed procedures for systematic and stepwise processing for interception of pests (Fig. 1): visual and stereo-binocular examination to intercept presence of smut and bunt balls, seed galls, ergot sclerotia, rust pustules, spores on the seed; washing test for rusts and downy mildews; blotter method for fungi, bacteria and infectivity, electron microscopy (EM), serological (ELISA) and molecular tests (PCR) to detect seed-borne pathogens including viruses. Infected materials are either incinerated or salvaged using various salvaging techniques depending on the category of pest(s) intercepted prior to release. As a result of quarantine processing it has been observed that seed-borne pests may cause losses in quality and germination of seed, distribution of new strains or physiological races of pest(s) along with the seeds and planting material to new geographical areas and development of epiphytotics. Therefore, critical

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Critical quarantine examinations with specialized tests are conducted to ensure the interception and identification of fungal, bacterial, viral pathogens, insects and mites, nematodes and weeds of quarantine importance associated with seeds and other planting materials.

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During quarantine processing, seeds and plant material is examined for the presence of unwanted weed seeds, plant debris, soil clods, insect and mite pests, plant parasitic nematodes and pathogens including fungi, bacteria, viruses, phytoplasma etc. (Bhalla, et al., 2018). The process of examining different number of samples and number of seeds per sample are different for bulk and small samples. However, the techniques used for detecting different pests are the same.

The external feeders and other incidental insect pests, infesting the seeds, are easily detected visually either by naked eye or with the help of magnifying glass or stereoscopic binocular microscope. Fungal infection is indicated by the presence of malformed seeds and fungal fructifications with the seed/ on seed surface. Presence of yellow discolouration around the hilum is suggestive of bacterial infection.

### Detection of insects and mites

Specialized/ advanced tests are used for detection of different groups of pests are:

- X-ray radiography is used to detect seeds infested with phytophagous chalcidoids, bruchids and certain other insect groups that do not exhibit any external symptoms on seed surface. Crops like pulses, oilseeds, tree species and some grasses are compulsorily subjected to X-ray radiography as far as germplasm import is concerned (Bhalla et al., 2002). On viewing the X ray on the screen with real-time X ray system, seeds showing insects are hand-picked and healthy seeds are released to the indentor.
- Transparency method is used for detecting infestation in small seeds like sesame. The seeds are boiled in lactophenol solution (lactophenol, lactic acid, distilled water and glycerine in the ratio of 2:2:2:1, respectively) for 1-2 hours depending on the hardness of the seeds. This renders them transparent to reveal insect infestation.

### Detection of fungal and bacterial pathogens

- Examination of suspension after seed washing is used for detecting surface-borne pathogens. The seeds are shaken in water and the resultant suspension is examined for spores of downy mildew, powdery mildew and some of the fungi under the compound microscope.



- Blotter method is used for detection of many fungal pathogens which are capable of producing mycelial growth and fruiting structures under incubation and also the bacterial pathogens. Seeds are placed on moist filter paper in plastic petriplates and incubated at 20 + 1°C under fluorescent tubes in alternating cycles of 12 hours light/darkness for 7 days growth of fungi and bacteria.

### Detection of seed-transmitted viruses

- Seeds known/suspected to carry seed-transmitted viruses are grown in insect-proof post-entry quarantine glass house/ environment controlled environment-controlled screen houses. Seedlings showing viral symptoms are uprooted and burned. Produce from only healthy plants is released to the indentors.
- Infectivity test is done to assay the presence of virus by inoculating leaf extracts of seedlings showing symptoms on indicator hosts. This method reveals the symptomless or latent infections of plants in grow-out tests.
- Enzyme-linked immunosorbent assay, a relatively simple, rapid and sensitive technique is used for simultaneous testing of a large number of samples.
- Leaf extract of infected seedlings is observed under transmission electron microscope, which reveals shape and size of virus particles.

### Detection of plant parasitic nematodes

- Soaking of seeds known/suspected to carry seed-borne nematodes in water overnight softens the seed coat which are then teased/crushed enabling the nematodes, if present, to come out in water. Examination of accompanying soil reveals the presence of viable nematodes especially ectoparasites and cysts of certain nematodes.

### Methodology of Salvaging Infested/ Infected/ Contaminated Material

Various methods are deployed for salvaging as given below:

- The soil clods, plant debris, weeds, discoloured, deformed and shrivelled seeds are mechanically cleaned by hand picking.
- Hot water treatment at various temperature and time combinations are used for eliminating pathogens like fungi, bacteria and nematodes. The treatment is given in hot water treatment tank fitted with heaters of different capacities, stirrer, thermostat and contact thermometer

for controlling the water temperature. All the samples of Brassica spp. are given mandatory hot water treatment to eradicate the seed-borne pathogens.

- X-ray radiography is used to separate insect-infested seeds (which do not have any external symptoms) from healthy ones. The seeds are laid in single layer and exposed to X-rays. On developing the X-ray film, the infested seeds can be easily distinguished and are hand-picked from the seed geometry. This method is not practical for use for bulk material, however, upon finding infestation this is combined with fumigation to salvage infested seeds.
- Fumigation is one of the most effective methods used in quarantine for eliminating insects, mites and nematodes done either at atmospheric pressure or under vacuum conditions. Atmospheric fumigation is done at normal air pressure in an air tight container using Aluminium phosphide @ 2 g/ cu m or ethylene dichloride- carbon tetrachloride mixture (3: 1) @ 320 mg/ l at 30°C for 48 hrs. Vacuum fumigation is done in especially designed fumigation chamber which helps in hastening the penetration of the fumigant through tightly packed material or internal infestation. The commonly used fumigants are ethylene oxide and carbon dioxide mixture, hydrogen cyanide gas and methyl bromide.
- Pesticidal treatment is the most practical method to use in quarantine for effective control of surface feeding insects, nematodes etc. Chemical seed dressing is generally given for eliminating seed-borne fungi and bacteria.
- For eliminating the externally seed-borne spores of safflower rust (*Puccinia carthamii*), the contaminated seeds are taken in a test tube containing ethyl alcohol (spirit) and a pinch of river sand and stirred with a mechanical stirrer for 30 seconds. The spores adhering to the seed surface get agitated and separate out in the alcohol.
- Chemically treated seed material is grown in isolation in post-entry quarantine isolation for one crop season for the detection of seed-borne pathogens. Healthy seeds from the uninfected plants are then released to the indentors.
- There are about 1300 seed-transmitted pests which infect a variety of plants. Besides, physical, chemical and mechanical methods, tissue culture technique is the safest for eliminating associated pests from the germplasm, wherever possible. Shoot tip culture is an efficient technique of raising pathogen-free plants from infected germplasm.

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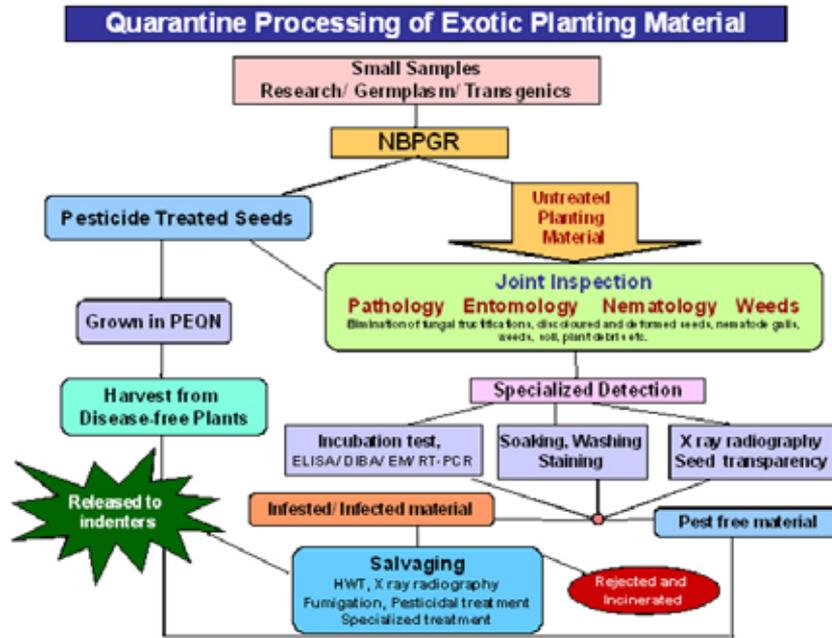


Fig. 1: Flowchart of quarantine processing of planting material.

### Interceptions in Imported Material

Over the years, during quarantine processing, a large number of pests have been intercepted in imported PGR and other research material. The significant interceptions made which are yet not reported from India include fungi like *Claviceps purpurea* in seeds of wheat, barley, *Peronospora manshurica* on soybean from several countries, *Fusarium nivale* on wheat, and barley from Germany, Italy, Hungary, Sweden and UK, *Uromyces betae* on sugarbeet from Belgium, Germany, Italy, UK and USA, *Phoma lingam* on Brassica from several countries, *Phomopsis longicolla* on *Glycine max* from USA, bacteria like *Xanthomonas campestris* pv. *campestris* on Brassica spp. from Canada, France, Pakistan, Sweden, Taiwan, UK and USA, viruses like Barley stripe mosaic virus on wheat from USA, Broad bean stain virus on *Pisum sativum* from Spain and *Vicia faba* from Syria and Bulgaria, Cowpea mottle virus on *V. subterranea* from Ghana and *V. unguiculata* from Philippines, Raspberry ring-spot virus on soybean from AVRDC (Taiwan), Sri Lanka, Thailand, USA, etc. and Cherry leaf roll virus on *Glycine max* from Taiwan, Sri Lanka, Thailand and USA and *Phaseolus vulgaris* from Colombia. Insects like *Acanthoscelides obtectus* in *Cajanus cajan* and *Phaseolus vulgaris* from Brazil, Colombia, Italy

and Nigeria, *Anthonomus grandis* in *Gossypium* spp. from USA, *Ephestia elutella* in *Macadamia* nuts and *Vigna* spp. from USA, *Quadrastichodella eucalyptii* in *Eucalyptus* from Australia and nematodes like *Heterodera schachtii* from Denmark, Germany and Italy, *Ditylenchus dipsaci*, *D. destructor*, *Rhadinaphelenchus cocophilus*, etc. in soil clods and plant debris, and weeds like *Cichorium spinosum* and *Echinochloa crus-gavonis*, which are not yet reported from India.

The pests intercepted can be categorized as:

- (i) those which are not known to occur in India;
- (ii) have different races/ biotypes/ strains not known to occur in India;
- (iii) are present on a new host or are from a country from where they were never reported before;
- (iv) an entirely new pest species hitherto unreported in science or
- (v) are reported to be present in India but with a wide host range

**Interceptions, especially of pests and their variability not yet reported from India signify the importance of quarantine in preventing the introduction of destructive exotic pests. The third and fourth category of pests are not expected in the sample as per the risk analysis which is literature-based and since no records are available on the pest/ host their presence is unexpected and important from quarantine view point.**

Interceptions, especially of pests and their variability not yet reported from India signify the importance of quarantine in preventing the introduction of destructive exotic pests. The third and fourth category of pests are not expected in the sample as per the risk analysis which is literature-based and since no records are available on the pest/ host their presence is unexpected and important from quarantine view point. The last category - pests with a wide host range are critical and could become invasive in case they find suitable biotic and abiotic environment (Khetarpal and Gupta, 2008). Such interceptions signify the success of quarantine, otherwise, these pests would have entered the country and played havoc with the plant biodiversity and agriculture.

It is clear that under the present international scenario, the quarantine specialists have a major role to play not only in promoting and facilitating the export and import in the interest of their respective nations but also in protecting the environment from the onslaughts of invasive alien species. The importance of quarantine has increased manifold in the WTO regime and adopting not only the appropriate technique but also the right strategy for pest detection and diagnosis would go a long way in ensuring pest-free exchange of germplasm and transparency in international exchange, and is considered the best strategy for managing transboundary movement of pests.



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# Wild Relatives Of Pigeonpea [Cajanus Cajan (L). Millspaugh] And Their Use In Its Genetic Improvement

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base and introgression of novel traits. The wild relatives of *Cajanus* serves as a stockpile of many economically important traits and these novel genes can be introgressed from the secondary and tertiary gene pool of *Cajanus* to the cultivated forms by using suitable breeding methods. Wild species of *Cajanus* serve as a potential source of resistance against insects like pod borers, pod fly and pod wasp and diseases like Fusarium wilt, Phytophthora blight and sterility mosaic.

## Introduction

Pigeon pea commands a high place in rainfed farming system as it is a hardy and drought tolerant crop. In India, during 2017-18 pigeon pea was annually grown on 4.43-million-hectare area with an annual production of 4.25 Mt million (Anonymous 2018). In India, pigeon pea can serve as an important source of food security as 64 % of the country's net sown area is under rainfed conditions. Breaking the yield plateau, reducing the maturity duration, creation of dwarf genotypes and development of resistant varieties against various biotic and abiotic stresses are the major challenges in the pigeon pea breeding. The domestication process results in a genetic bottleneck due to which a very few gene combinations get accumulated in the cultivated species of pigeon pea and these cultivars possess only a small portion of the overall genetic diversity present within the gene pool (Kassa et al 2012). This narrow genetic diversity can be broadened by the utilization of wild relatives of crop plants (WRCPs). Wild species especially the wild relatives of crop plants (WRCPs) acts as a reservoir of genes for yield, resistance to various biotic and abiotic stresses and quality traits. The wild species conserves a large proportion of untapped genes that have potential to be utilized in crop improvement. Pazhamala et al (2015) in their review reported that the germplasm of pigeonpea is represented by 13,771 accessions deposited at the ICRISAT, India genebank (Gowda et al 2013), 11,221 accessions collected at National Bureau of Plant Genetic Resources (NBPGR), India (Singh et al 2014), 4,116 accessions at U.S. Department of Agriculture (USDA), USA and 1,288 accessions at Kenya Agricultural Research Institute's National Genebank of Kenya (KARI-NGBK), Kenya (Singh et al 2013).

The cultivated varieties of pigeon pea do not possess broad genetic base and hence the use of wild relatives in the breeding programme would help to broaden its genetic base and introgression of novel traits. The wild relatives of *Cajanus* serves as a stockpile of many economically important traits and these novel genes can be introgressed from the secondary and tertiary gene pool of *Cajanus* to the cultivated forms by using suitable breeding methods. Wild species of *Cajanus* serve as a potential source of resistance against insects like pod borers, pod fly and pod wasp and diseases like Fusarium wilt, Phytophthora blight and sterility mosaic. Breaking the yield plateau, reducing the maturity duration, creation of dwarf genotypes and development of resistant varieties against various biotic and abiotic stresses are the major challenges in the

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Pigeon pea crop is suffered from many abiotic stresses such as drought, salinity, waterlogging, soil salinity and aluminium toxicity as well as abiotic stresses like insects (Pod Borer and Pod Fly) and diseases like Fusarium wilt, Phytophthora blight and Sterility mosaic disease

pigeonpea breeding. Wild species of pigeonpea such as *C. scarabaeoides*, *C. acutifolius*, *C. albicans*, *C. lineatus*, *C. Platycarpus* and *C. sericeus* acts as storehouse of several useful genes viz., genes for biotic and abiotic stress resistance, genes for high seed protein content, genes for cytoplasmic male sterility and superior morphological traits. Pigeon pea crop is suffered from many abiotic stresses such as drought, salinity, waterlogging, soil salinity and aluminium toxicity as well as abiotic stresses like insects (Pod Borer and Pod Fly) and diseases like Fusarium wilt, Phytophthora blight and Sterility mosaic disease Table 1. The wild relatives of pigeonpea viz., *C. scarabaeoides*, *C. acutifolius*, *C. albicans*, *C. lineatus*, *C. Platycarpus* and *C. sericeus* contains genes that confers resistance against these stresses and hence these wild relatives can be used in hybridization programmes as a donors.

**Table 1: Major Abiotic and Biotic Stress of Pigeonpea in India**

S.No.	Abiotic stress	Cause
1.	Waterlogging	Prolonged water saturation
2.	Drought stress	Prolonged period of low rainfall
3.	Low temperature stress	During winter months (December-January)
4.	Soil salinity stress	Salt accumulation in soil surfaces
5.	Aluminium toxicity	Aluminium toxicity occurs in acid soil (pH<5.5)

	Biotic Stress	Cause (Insects)
1.	Pod Borer	Helicoverpa armigera
2.	Spotted Pod Borer	Maruca vitrata
3.	Pod Fly	Melanagromyza obtuse
	Biotic Stress	Cause (Fungi)
1.	Phytophthora Blight Disease	Phytophthora drechsleri Tucker f. sp. Cajani
2.	Fusarium Wilt Disease	Fusarium udum
3.	Sterility Mosaic Disease	Pigeonpea sterility mosaic virus (PPSMV)

The genus *Cajanus* is very rich in terms of diversity of germplasm. There are total 32 species present in the genus *Cajanus* out of which 18 species are endemic to Asia, 13 to Australia and one to West Africa.

### Gene Pool of Pigeonpea

The genus *Cajanus* is very rich in terms of diversity of germplasm. There are total 32 species present in the genus *Cajanus* out of which 18 species are endemic to Asia, 13 to Australia and one to West Africa. The gene pool of *Cajanus* comprised of primary, secondary, tertiary and quaternary pools. These pools can be used to trap valuable genes by application of various gene transfer methodologies.



1. **Primary gene pool:** The primary gene pool includes the species that are easy to cross with cultivated types and produces fertile hybrids on crossing. The primary gene pool of *Cajanus* chiefly consists of cultivated species and its landraces. For most of the economically important traits the genus *Cajanus* contains large phenotypic variation within primary gene pool but the molecular studies have shown that there is limited genetic variability in the germplasm (Saxena et al 2016). Kumar et al (2003), studied the diversity among the parents used in developing the pigeonpea cultivars and concluded that 50% of cultivars had 10 parents in common.
2. **Secondary Gene pool:** The species in secondary gene pool includes the distantly but cross compatible wild related species, leading to partly fertile cross-progenies. There are nearly ten species in the secondary gene pool of the *cajanus* i.e. *cajanifolius*, *lineatus*, *lanceolatus*, *laticepalus*, *albicans*, *reticulatus*, *sericeus*, *scarabaeoides*, *trinervius* and *acutifolius* (Mallikarjuna et al 2011). Wild species present in this pool are storehouse of a number of genes that can be used for development of disease and pest-resistant lines, dwarf plant stature, cytoplasmic nuclear male sterile systems (CMS) and high protein lines (Mallikarjuna and Saxena 2005).
3. **Tertiary gene pool:** This gene pool is most difficult to exploit as it contains distantly related species or unrelated taxa. This pool of *cajanus* contains species like *goensis*, *heynei*, *kerstingii*, *crassus*, *mollis*, *rugosus*, *volubilis*, *platycarpus*, *niveus*, *grandiflorus*, *crassicaulis*, *elongates*, *villosus*, *convertiflorus*, *visidus*, *aromaticus*, *pubescens*, *cinereus*, *marmoratus*, *mareebensis* and *lanuginosus* (Mallikarjuna et al 2011).
4. **Quaternary gene pool:** It includes genus like *Flemingia*, *Rhynchosia*, *Dunbaria*, *Erisema*, *Paracalyx*, *Adenodolichos*, *Bolusafra*, *Carissoa*, *Chrysoscias* and *Baukea* (Mallikarjuna et al 2011).

## Wild relatives of pigeonpea as a source of resistance genes for various Abiotic and Biotic stresses

### Wild relatives of pigeonpea for Insect resistance

#### Morphological and Biochemical characters in wild relatives associated with insect resistance:

Wild relatives of *Cajanus* possess certain morphological as well as biochemical characters that make it difficult for the insect pests to survive on them. The morphological characters like toughness of pod, cell wall lignification and non-glandular trichomes (Type C and D) on the surface of pods of wild relatives of *Cajanus* have been reported to be associated with resistance to *H. armigera*.

The morphological characters like toughness of pod, cell wall lignification and non-glandular trichomes (Type C and D) on the surface of pods of wild relatives of *Cajanus* have been reported to be associated with resistance to *H. armigera*. The traits like determinate plant type, clustered pods and dense canopy favours the attack of pod borers (*H. armigera* and *M. vitrata*) while the genotypes having non-clustered, small pods with seeds tightly fitted to the pod wall are less susceptible to *H. armigera* (Sharma and Franzmann 2000; Sharma et al 1999,).

The traits like determinate plant type, clustered pods and dense canopy favours the attack of pod borers (*H. armigera* and *M. vitrata*) while the genotypes having non-clustered, small pods with seeds tightly fitted to the pod wall are less susceptible to *H. armigera* (Sharma and Franzmann 2000; Sharma et al 1999,). Some workers have reported that oviposition non-preference and antibiosis is associated with resistance of pod borer in accessions of wild species of *Cajanus* (Dodia et al 1996). *C. scarabaeoides* and *C. acutifolius* have nonglandular trichomes and hence they are not preferred by females of pod borers for egg laying. The (Type A) trichomes present in the *C. cajan* acts as attractants or phagostimulants for *H. armigera* (Green et al. 2003) while the dense nonglandular trichomes type C on pods of wild pigeonpea act as a physical barrier to the feeding by the young *H. armigera* larvae (Romeis et al. 1999). Chemicals extracted in acetone from the pod surface of *C. scarabaeoides* results in feeding inhibition, whereas compounds extracted from the pod surface of *C. cajan* in methanol act as phagostimulants (Romeis et al. 1999; Green et al. 2003). Sharma et al (2009) reported heavy egg-laying on *C. cajanifolius* (Cultivated Spp.) however the *Helicoverpa* shows oviposition non-preference in *C. scarabaeoides* (Wild Spp.). The seeds of pigeonpea contain proteinaceous inhibitors (PIs) which serve as a mechanism of defence against the herbivores, however with the course of time *H. armigera* has overcome the effect of host plant PIs (Ranjekar et al 2003). However, PIs present in the wild relatives of pigeonpea, have shown a high level of resistance for *Helicoverpa* and they will be more effective as inhibitors of proteases in its gut and thus help in controlling the insect attack (Parde et al 2012). High concentration of phytoalexin known as Stilbene is related to resistance towards *H. armigera* in pigeonpea (Green et al 2003). The amount of total phenols and tannins in the podwall of pigeonpea are negatively associated with the damage of pod fly (*M. obtusa*). In pigeonpea, the podwall's protein content is associated with susceptibility, whereas the total sugars are associated with resistance to *M. obtusa* in pigeonpea.

**Table 2: Wild relatives as a source of resistance genes for various biotic stresses (Insects)**

S.No.	Insects	Source of resistance	References
1.	<i>Helicoverpa armigera</i> (Pod Borer)	<i>C. acutifolius</i> , <i>C. albicans</i> , <i>C. cajanifolius</i> , <i>C. platycarpus</i> , <i>C. reticulatus</i> , <i>C. scarabaeoides</i> , <i>C. sericeus</i> <i>R. aurea</i> and <i>Flemingia bracteata</i>	Singh et al 2013 Rao et al 2003, Sharma et al (2009) Sujana et al (2008) and Mallikarjuna et al (2007)

S.No.	Insects	Source of resistance	References
2.	Melanagromyza obtuse (Pod Fly)	C. acutifolius, C. albicans, C. cajanifolius, C. lineatus, C. reticulatus, C. sericeus, C. scarabaeoides and C. Platycarpus	Sharma et al (2003)
3.	Tanaostigmodes cajaninae (Pod Wasp)	C. scarabaeoides, R. bracteata, C. albicans and F. stricta	Sharma et al (2003)
4.	Bruchid	C. acutifolius, C. platycarpus and C. scarabaeoides	Singh et al 2013

## Wild relatives of pigeonpea for Disease resistance

### Morphological and Biochemical Characters in wild pigeonpea associated with disease resistance:

Although a number of sterility mosaic resistant varieties viz., Bahar, Sharad, Asha, BSMR175, BSMR 736 and Fusarium Wilt resistant varieties viz., Maruti, ICPL87119, BDN 1, BDN 2, C11, NP(WR15), Narendra Arhar-1, ICPL 87 (Pragati), Jawahar (KM 7) had been already developed in pigeonpea but as the pathogens are continuously evolving hence new source of resistant need to be discover. CWR can be used as a very important source of candidate genes against these diseases. Sterility mosaic disease (SMD) is a viral disease of pigeonpea caused by a virus called as Pigeonpea Sterility Mosaic Virus (PPSMV), this virus is transmitted by eriophyid mite (*Aceria cajani*). The eriophyid mites inhabit the lower surface of leaflets of pigeonpea. The dense trichomes present on the lower leaf surface of cultivated genotypes (*Cajanus cajan*) provide a vital microclimate for survival and multiplication of eriophyid mites whereas on the lower leaf surface of wild *Cajanus* species trichomes were very sparse. This physical factor may be responsible for the poor survival of mites on the wild species of *Cajanus*. Some SMD resistant pigeon pea genotypes have a thicker leaf cuticle and epidermal cell wall that checks the stylet of mite from reaching to epidermal cells. Fusarium wilt caused due to a soil borne fungus *Fusarium udum* is one of the most severe disease of pigeonpea in Indian subcontinent (Singh et al. 2013). Murthy, 1975 in his study found that resistant varieties of pigeonpea have a higher content of total sugars, amino acids and phenols but a lower content of phenylalanine. A resistant variety of pigeonpea contains caffeic, chlorogenic acids and unidentified phenolic compounds that inhibit spore germination. Cysteine was also effective in counteracting fungal infection by chelating ferric ions and thereby inactivating *Fusarium* toxin.

**Sterility mosaic disease (SMD) is a viral disease of pigeonpea caused by a virus called as Pigeonpea Sterility Mosaic Virus (PPSMV), this virus is transmitted by eriophyid mite (*Aceria cajani*).**

**Fusarium wilt caused due to a soil borne fungus *Fusarium udum* is one of the most severe disease of pigeonpea in Indian subcontinent**

**Table 3: Wild relatives as a source of resistance genes for various biotic stresses (Diseases)**

S.No.	Diseases	Source of resistance	References
1.	Phytophthora Blight Disease	<i>C. platycarpus</i>	Mallikarjuna et al 2006
2.	Fusarium Wilt Disease	<i>C. scarabaeoides</i> <i>C. lineatus</i> and <i>C. lanceolatus</i> ,	Singh et al., 2013
3.	Sterility Mosaic Disease	<i>C. albicans</i> and <i>C. scarabaeoides</i> <i>C. lineatus</i> , <i>C. sericeus</i> , <i>C. platycarpus</i> , <i>C. crassus</i> and <i>C. volubilis</i>	Kulkarni et al 2003 and Kumar et al 2005 and Singh et al., 2013

### Wild relatives of pigeonpea for Abiotic stress resistance

Wild relatives of pigeon pea also had genes providing tolerance against several abiotic stresses. Among abiotic stress soil salinity is a major constraint. Some cultivated varieties such as 'C 11' (Chauhan 1987), 'UPAS 120' had been identified as tolerant to salinity. The wild relatives of pigeonpea viz., *C. scarabaeoides*, *C. albicans* and *C. platycarpus* were found to show tolerance towards salinity. The main factors present in wild relative that confers tolerance toward salinity were Na and Cl retention in the roots and limited translocation to the shoots, high K selectivity and maintenance of transpiration rate under saline conditions. Due to its deep tap root system pigeon pea is able to extract water from deep level of soil and hence is a drought tolerant crop. But under severe water stress it may develop symptoms of drought. The parameters like dehydration tolerance, Relative Water Content and OA (Osmotic adjustment) are very important to tackle drought conditions. While Conducting a breeding programme for developing drought tolerant varieties due emphasis should be given to traits like pods/plant, seeds/pod, seed size and seed yield/plant under actual water deficit condition. In case of waterlogging the parameters such as lenticels development, more root biomass and adventitious roots are helpful in combating the water stress condition. In case of Aluminium toxicity, the exclusion of aluminium from the roots is responsible for providing tolerance. Choudhary et al., 2011 suggested that more studies will be needed to screen the wild relatives like *Cajanus scarabaeoides* and *C. platycarpus*, as these wild forms may have genes for tolerating aluminium toxicity. Table 4 below is showing the wild relatives having desirable genes for abiotic stresses.

deep tap root system pigeon pea is able to extract water from deep level of soil and hence is a drought tolerant crop. But under severe water stress it may develop symptoms of drought.

**Table 4: Wild relatives as a source of resistance genes for various abiotic stresses**

S.No.	Abiotic stress	Source of resistance	References
1.	Waterlogging	<i>C. scarabaeoides</i>	Krishnamurthy et al 2012
2.	Drought stress	<i>C. scarabaeoides</i> , <i>C. acutifolius</i> , <i>C. albicans</i> , <i>C. lanceolatus</i> , <i>C. lineatus</i> , <i>C. sericeus</i> , <i>C. trinervius</i>	Upadhyaya 2006 and Singh et al 2013
3.	Soil salinity stress	<i>C. platycarpus</i> , <i>C. acutifolius</i> , <i>C. albicans</i> , <i>C. cajanifolius</i> , <i>C. lineatus</i> , <i>C. scarabaeoides</i> , <i>C. sericeus</i>	Subbarao et al 1990 and Singh et al 2013

### Wild relatives as a source of quality related traits

Various quality related traits in pigeonpea like seed protein content and high content of sulphur containing amino acids (methionine and cysteine) as well as productivity related traits can be improved in cultivated pigeonpea by using wild relatives.

**Table 5: Wild relatives as a source of productivity and quality related traits**

S.No.	Traits	Wild Relatives	References
1.	High Seed Protein Content (> 30%)	<i>C. scarabaeoides</i> , <i>C. sericeus</i> and <i>C. albicans</i>	Reddy et al 1997 and Mallikarjuna et al 2011
2.	High content of sulphur containing amino acids (methionine and cysteine)	<i>Flemingia</i>	Remanandan (1980)
3.	Annual Habit	<i>C. platycarpus</i>	Singh et al 2013
4.	Improved Plant type	<i>C. sericeus</i>	
5.	Long fruiting branches	<i>C. sericeus</i>	
6.	Dwarfness	<i>C. cajanifolius</i>	
7.	Large pods and leaf	<i>C. grandiflorus</i>	
8.	High seeds /pod	<i>C. goensis</i> , <i>C. mollis</i>	
9.	High pod setting	<i>C. albicans</i> , <i>C. platycarpus</i> , <i>C. scarabaeoides</i> , <i>C. sericeus</i>	

## Wild Relatives as source of Cytoplasmic Male Sterility

In pigeonpea, eight different cytoplasm (A1–A8) have been identified as sources of male sterility. Of these, A2 and A4 cytoplasm derived from *Cajanus scarabaeoides* and *Cajanus cajanifolius*, respectively, have been optimized for regular use in hybrid breeding

**Table 6: Wild relatives as a source of for Cytoplasmic male sterility**

S.No.	Type of CMS	Wild Relatives	References
1.	A1	<i>C. sericeus</i>	Ariyanayagam et al 1995
2.	A2	<i>C. scarabaeoides</i>	Tikka et al 1997; Saxena and Kumar 2003
3.	A3	<i>C. volubilis</i>	Wanjari et al 2001
4.	A4	<i>C. cajanifolius</i>	Saxena et al 2005
5.	A5	<i>C. acutifolius</i>	Mallikarjuna and Saxena 2005
6.	A6	<i>C. lineatus</i>	-
7.	A7	<i>C. platycarpus</i>	-
8.	A8	<i>C. reticulatus</i>	Saxena 2013

## Conclusion

It can be concluded that the wild relatives of *Cajanus* are a reservoir of several desirable genes viz., genes for biotic and abiotic stress resistance, genes for high seed protein content, genes for cytoplasmic male sterility and superior morphological traits. By using proper breeding strategies these genes can be introgressed in the cultivated pool of the *Cajanus*. Hence, the wild relatives are very important assets for plant breeders.

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# Genetic resources of maize and their utilization for Crop Improvement

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Global warming has led to aberrant changes in weather conditions worldwide which have predisposed crop plants to various biotic and abiotic stresses leading to reduction in crop yields. On the other hand, increasing human population necessitates corresponding increase in production of food grains in order to meet growing food demands. The demand for maize in developing countries will double between now and 2050. In order to meet this increasing maize demand it will become essential to enhance maize yield and prevent yield losses due to biotic and abiotic stresses. Breeding for climatic resiliency in maize would require maize genetic resources with wide genetic variability which can act as donor of resistant alleles. Conservation, evaluation and characterization of maize genetic resources therefore becomes inevitable in view of the increasing demand of maize and increased frequency of biotic and abiotic stresses.

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## Introduction

Maize (*Zea mays* L.) is a monoecious crop which originated in the highlands of Mexico approximately 8700 years ago and is one of the most widely cultivated crops in the world after wheat and rice. Due to its high popularity it is often referred to as “The queen of cereals”. It is grown in more than seventy countries including 58 developing and 15 developed countries. Along with rice and wheat it provides 60% of the world’s energy intake. With an area of 187.95 m ha and a production of 1060.10 m tonnes, maize ranks third in world after wheat and rice. It contributes 39% of grain production globally. The highest maize production comes from the United States of America (USA) i.e., 36% of the world production and maize serves as the driver of US economy. In Indian context maize is grown throughout the year but is predominantly a kharif crop covering 85% of the total area under cultivation in the season. With an area of 8.69 m ha, a production of 21.81 m tonnes and 2509 Kg/ha productivity maize ranks fifth in area after rice, wheat, jowar and bajra, fourth in production and third in productivity. Highest maize production is harvested in the state of Karnataka followed by Madhya Pradesh and Bihar. World population is increasing at an alarming rate. With the current rate of population increase the demand for food alone in the developing countries is predicted to increase by 1.3 % annually till 2020. The demand for maize in developing countries will double between now and 2050. In order to meet this increasing maize demand it will become essential to enhance maize yield and prevent yield losses due to biotic and abiotic stresses. Breeding for climatic resiliency in maize would require maize genetic resources with wide genetic variability which can act as donor of resistant alleles. Conservation, evaluation and characterization of maize genetic resources therefore becomes inevitable in view of the increasing demand of maize and increased frequency of biotic and abiotic stresses owing to rapid and aberrant climate change.

## Plant Genetic Resources (PGRs)

According to an International Undertaking on Plant Genetic Resources, plant genetic resource usually refers to either reproductive or vegetative propagating material that includes primitive cultivars (landraces); obsolete cultivars; cultivated varieties in current use; newly developed varieties; wild and weed species and special genetic stocks including mutants and breeding lines. Moxted and Kell (2003) defined PGRs as the genetic material having value for present and future generations. PGRs acts as storehouse of rare genetic traits required to cope with biotic and abiotic stresses. Conservation of PGRs have become important in view of rapid erosion of genetic diversity due to modification and destruction of natural habitat and replacement of genetically variable traditional landraces with genetically uniform crop varieties. PGRs act as a link between genetic diversity and its utilization by humans and therefore needs to be conserved so that they continue to act as source of beneficial

alleles in future. Conservation of PGRs can be done by either in situ or ex situ conservation practices. However, a balance between the two conservation approaches is essential for effective germplasm conservation.

## Wild relatives as an integral component of maize PGRs

Maize comprises of eight genera, among these Coix, Polytoca, Schlerachne, Trilobachene and Chinonachne are comparatively less important ones while three genera viz., Zea, Euchalaena and Tripsacum are most important. Zea and Tripsacum belonging to subtribe Tripsacinae, tribe Andropogoneae, and subfamily Panicoideae of family Poaceae constitutes the genera that comprise the genepool of modern maize and presents the genetic diversity available for crop improvement either naturally through hybridisation or by use of artificial techniques as embryo rescue. The teosinte have been split into three subspecies viz., *Z. mays huehuetenangensis*, *Z. mays mexicana*, *Z. mays parviglumis*, all of which are diploid and can readily cross with maize, and three species viz., *Z. luxurians*, *Z. diploperennis* and *Z. perennis* which shows increasing incompatibilities with maize. Balsas teosinte is considered the closest wild relative of modern maize (Galinat, 1988). All of the species within genus *Zea* except *Zea mays* constitutes the secondary gene pool of maize while genus *Tripsacum* belongs to the tertiary gene pool.

### Use of maize wild relatives for crop improvement

#### Insect tolerance

A number of field and storage insects' attacks maize and is responsible for reduced yield worldwide. In India there are around 250 insect and mites that infest maize crop however most of them are of minor significance with only a dozen causing widespread damage and requiring control measures. Major insects causing maize yield loss in India includes maize stem borer (*Chilo partellus*), pink stem borer (*Sesamia inferens*), two species of shoot fly, *Atherigona nuquii* and *Atherigona soccata*, armyworm (*Mythimna seprata*), maize cob borer (*Helicoverpa armigera*) and maize aphid (*Rhopalosiphum maidis*). Recently an American pest fall armyworm (*Spodoptera frugiperda*) have also been found to causes economic losses in maize crop at mild to alarming levels. Besides field, insects also causes spoilage in storage by feeding on grains, damaging kernel, destroying the germ portion and encouraging growth of fungus due to increased temperature of the grains. Rice weevil, khapra beetle, lesser grain borer and Angoumois grain moth are major storage pest infesting maize grains in warehouses. Development of varieties resistant to insect attack is an economically viable alternative compared to pest management by use of insecticides. Wild relatives of maize are reported to be storehouse of a number of traits providing resiliency to insect attack. Resistance to insect attack is mainly a result of non-preference, antibiosis, tolerance or release of secondary metabolites which acts as an attractant to

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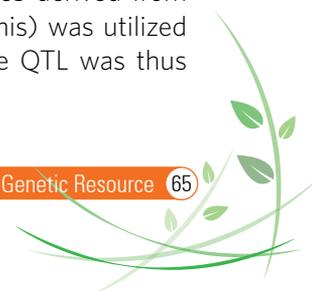
Certain morphological features of crop wild relatives are also responsible for non-preference of these genotypes by insects for feeding and oviposition. Balsas teosinte for example have certain physical barriers that makes them less desirable by insects.

Fall Armyworm (FAW) (*Spodoptera frugiperda*) did not preferred balsas teosinte for oviposition.

natural enemies of insects. Maize plants in response to herbivory by insects are reported to emit certain volatile substances (VOCs) which acts as an attractant to insect natural enemies. The type of VOCs released depends on which part of plant is damaged by herbivory and in turn it also decides the type of natural enemies attracted. Damage to foliar parts due to attack by lepidopterans released a blend of indole and a number of mono- and sesquiterpenes. These were reported to attract parasitic wasp as *Meteorus laphygmae* and *Cotesia marginiventris* which were predatory on FAW. Spotted stalk borer oviposition triggered the release of (E)-4,8-dimethyl-1,3,7-nonatriene from teosinte foliage which attracted both egg (*Trichogramma bourneiri*) and larval (*Cotesia sesamiae*) parasitoids. A volatile herbivory induced plant signal in maize is the release of (E)- $\beta$ -caryophyllene, which strongly attracts an entomopathogenic nematode in response to damage by larvae of beetle *Diabrotica virgifera virgifera*. In an experiment it was concluded that the nearest wild relative of maize, teosinte (*Zea mays parviglumis*) releases larger amount of this substance in response to feeding by caterpillar compared to maize genotypes. Certain morphological features of crop wild relatives are also responsible for non-preference of these genotypes by insects for feeding and oviposition. Balsas teosinte for example have certain physical barriers that makes them less desirable by insects. Increased leaf toughness compared to maize genotypes was one of the reasons leaves hoppers (*Dalbulus maidis*) and Fall Armyworm (FAW) (*Spodoptera frugiperda*) did not preferred balsas teosinte for oviposition. Enhanced pubescence as measured by increased density of leaf trichomes was another reason that prevented damage by fall armyworm in teosinte species however it provided little protection against *D. maidis* oviposition. Beside physical barriers against insect damage, wild relatives also tend to possess certain toxic chemicals that makes them unsuitable for consumption by herbivore insects. Enhanced expression of RP1, wip and chitinase genes in balsas teosinte was responsible for reduced damage by FAW. Similarly metabolites present in leaf extracts of *Z. diploperennis* had an adverse effect on growth of FAW larvae along with increase in mortality rate. Higher concentration of benzoxazinoids (BXs) in *Z. mays* ssp. *Mexicana* compared to maize was responsible for greater resistance against maize spotted stalk borer (MSSB). Beside teosinte another distant relative of maize *Tripsacum dactyloides* have also been reported to be donor of a gene which is responsible for imparting rootworm resistance to corn genotypes. *Tripsacum* was also reported to be donor of maize weevil (*Sitophilus zeamais*) resistance.

## Disease tolerance

Quantitative Trait Loci (QTLs) responsible for providing resistance against a number of diseases were discovered in maize wild relatives. A study was conducted a study where a population of near isogenic lines derived from cross between maize and teosinte (*Zea mays* ssp *parviglumis*) was utilized for QTL mapping of gray leaf spot (GLS). A GLS resistance QTL was thus



identified in bin 4.07. It was reported that teosinte can also be used as donor of resistance to corn smut disease. *Z. diploperennis* was reported to be a source of resistance to southern corn leaf blight, northern corn leaf blight and corn leaf spot disease. Maize × *Z. diploperennis* crosses were studied using genomic in situ hybridization identified specific segments derived from *Z. diploperennis* imparting resistance to before mentioned three fungal diseases. A higher level of resistance was reported against a number of viral and mycoplasmal diseases of maize viz., maize chlorotic mottle virus, maize bushy stunt mycoplasma, maize streak virus, maize stripe and rayado fino virus and maize chlorotic dwarf virus in *Z. diploperennis*. *Z. mays* ssp. *Mexicana* was reported to be donor for *Fusarium* and downy mildew resistance. *Tripsacum dactyloides* L. is also reported to be source of many resistance alleles which when transferred into maize genetic background have historically helped to overcome various disease epidemics in maize. One of the example was the transfer of blight resistant alleles into commercial corn lines which resolved the problem of corn blight in USA. The introgression of Ht3, a northern leaf blight resistance genes and Rp1td, a novel rust resistance genes from eastern Gama grass into maize genetic background remains some other success stories of utilization of wild relatives to breed for disease resiliency in crop plants. Introgression from *Tripsacum* have also been reported to breed for *Helminthosporium* and *Puccinia* resistance in maize

## Weed tolerance

*Striga hermonthica* is a root parasite that affects both maize and sorghum cultivation around the world. Strigolactones are produced by the host plant that are perceived as germination signals by seeds of *S. hermonthica*. Resistance to *S. hermonthica* have been reported to be of qualitative nature in maize. However wild relatives of maize i.e., *Z. diploperennis* and *Tripsacum* have been reported to be resistant to parasitism by *S. hermonthica*. The mechanism of resistance of *Z. diploperennis* involves preventing attachment of germinating *S. hermonthica* to its roots and resisting the penetration of roots by weed haustoria. While *Tripsacum* is said to produce chemical signals that prevents development of weed haustoria on its roots and the resistance is reported to be quantitatively inherited. A wild relative of maize, *Tripsacum*, represents an untapped genetic resource for abiotic and biotic stress resistance and for apomixis, a trait that could provide developing world farmers access to hybrid technology.

## Conclusion

As climate changes and overall temperature grows up, inconsistent weather patterns and frequency of extreme weather events also surges at the global scale. It was found that 41% of maize yield variation in different corn belts was mainly due to climate with the climate variability on yield being as high as 60-75% in USA and China. The global movement of seeds, soil, and

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**It was found that 41% of maize yield variation in different corn belts was mainly due to climate with the climate variability on yield being as high as 60-75% in USA and China. The global movement of seeds, soil, and pests increases the pace of development of new production threats across global production areas.**

pests increases the pace of development of new production threats across global production areas. There is a wealth of genetic diversity retained in wild relatives of various crops, much of which remains to be discovered. Modern genetic and breeding technologies, along with big data analytics, will be crucial to support identification of germplasm that consist of desirable alleles from non-elite sources as wild relatives and landraces. The identification of useful germplasm involves genotyping and phenotyping and assessing performance of germplasm under a number of edaphic and climatic conditions. The swift improvement of biotechnological tools as diverse omics approaches has resulted in promising advances and there is no qualm that it will become routine in plant breeding programs. Advanced biotechnologies, such as genome editing and cisgenesis/intragenesis, are continuously being refined and will increase the demand of genetic diversity retained in wild relatives to breed for sustainability of agriculture. In order to ensure availability of maize and maize wild relative germplasm, it needs to be conserved.

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